Original Paper

Andrographolide Attenuates LPS-Induced Cardiac Malfunctions Through Inhibition of I κ B Phosphorylation and Apoptosis in Mice

Jinlong Zhang^a Didi Zhu^b Yulin Wang^a Yunfeng Ju^a

^aDepartment of Cardiology, The First People's Hospital of Yancheng Affiliated with Nantong University, Yancheng, China, ^bDepartment of Cardiology, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China

Key Words

Cardiac malfunction • LPS • Andrographolide • Apoptosis

Abstract

Background/Aims: Cardiac malfunction is a common complication in sepsis and significantly increases the mortality of patients in septic shock. However, no studies have examined whether andrographolide (And) reduces LPS-induced myocardial malfunction. **Methods:** Left ventricular systolic and diastolic functions were examined using echocardiography. TNF- α and IL-1 β protein levels were detected by an enzyme-linked immunosorbent assay (ELISA). NO oxidation products were determined using Griess reagent. Protein expression levels of inhibitors of NF- κ B (I κ B) and phospho-I κ B were determined via Western blot. Oxidative injury was determined by measuring myocardial lipid peroxidation and superoxide dismutase activity. Cardiac apoptosis was examined by terminal deoxynucleotidyl transferase-mediated dUTP nickend-labeling (TUNEL) and cardiac caspase 3/7 activity. **Results:** And blunted LPS-induced myocardial malfunctions in mice. LPS induced TNF- α , IL-1 β , and NO production as well as I- κ B phosphorylation. Cardiac apoptosis was attenuated via incubation with And, but the extent of oxidative injury remained unaffected. **Conclusion:** And prevents LPS-induced cardiac malfunctions in mice by inhibiting TNF- α , IL-1 β , and NO production, I κ B phosphorylation, and cardiac apoptosis, indicating that And may be a potential agent for preventing myocardial malfunction during sepsis.

© 2015 The Author(s)
Published by S. Karger AG, Basel

Introduction

The systemic inflammation of sepsis is initiated by pathogen-associated molecules, such as lipopolysaccharide (LPS), and is a leading cause of death in the intensive care unit [1, 2]. Recent progress has been made in the management of sepsis, but the mortality of sepsis is still high [2]. Myocardial malfunction is a common complication of sepsis inpatients

Yunfeng Ju

Department of Cardiology, The First People's Hospital of Yancheng Affiliated with Nantong University, Yancheng 224001, Jiangsu Province, (China)
E-Mail yfju01@sina.com

or in animal models of LPS-induced endotoxemia and 40% of patients with sepsis develop cardiac malfunction characterized by ejection fraction reductions, biventricular dilatations, and reduced responses to fluid administration [3, 4]. Emerging evidence has demonstrated that LPS-induced cardiac malfunction is mediated by cardiac apoptosis as well as nuclear factor-Nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B)-modulated over-expression of multiple proinflammatory mediators, including IL-1 β , TNF- α , and NO [4-8]. Cardiac abnormalities have been found to be reversible in surviving patients, but not in non-survivors [9], suggesting that myocardial malfunction significantly contributes to the mortality of patients in septic shock. In addition, in septic patients, the occurrence of myocardial malfunction significantly increases mortality from 20% to 70% ~ 90% [10]. Therefore, the development of effective approaches for attenuating cardiac malfunction during sepsis is imperative.

Andrographis Paniculata has been widely used as folk medicine in China for the treatment of viral infection, diarrhea, dysentery and fever for hundreds of years. Additional hepatoprotective, antihypertensive, antipyretic, antimalarial, and antithrombotic effects have also been reported, and Andrographis Paniculata has been used in antidotes for snake bites. Moreover, andrographolide (And), extracted and purified from Andrographis Paniculata, is currently prescribed for treatment of inflammation-related diseases, such as laryngitis, upper respiratory tract infection, rheumatoid arthritis, and cancer [11-13]. Recent studies have revealed that this herb also has cardiovascular effects [14-16], and And has been shown to protect hearts against ischemia/reperfusion [17]. These findings indicate that And may be a favorable alternative to cardiovascular therapy. And protects against LPS-induced injury by inactivating NF- κ B [18-25]. NF- κ B plays a pivotal role in the pathogenesis of inflammation, prompting drug design for the treatment of human inflammatory disease to focus on inhibiting NF- κ B activation [26].

However, thus far, no reported data has suggested that And prevents LPS-induced cardiac malfunction *in vivo*.

Materials and Methods

Animals and experimental design

All procedures for animal use were approved by the Committee on the Ethics of Animal Experiments of the First People's Hospital of Yancheng. Male BALB/c mice (6-8 weeks old, 22-25 g) were supplied by the Yangzhou experimental animal center. The mice were kept in 26°C temperature controlled room and given free access to food and water. Animals underwent an acclimatization period of at least 7 days before use in our study. The mice were then randomly divided into four groups: control, LPS, And+ LPS and And alone. Distilled water (0.1 mL/10 g body weight) or And (10 mg/kg; Sigma) was administered intragastrically once a day for 7 days. One hour after intragastrical treatment on day 7, normal saline (0.2 mL/10 g bodyweight) or LPS (*Escherichia coli*, 055:B5; Sigma; 20 mg/kg, 0.2 mL/10 g body weight) was injected intraperitoneally.

Echocardiographic examinations

After LPS administration, echocardiographic evaluation was performed using a commercially available echocardiograph (Vevo2100, VisualSonics, Canada). M-mode and Doppler echocardiography were performed according to previously described methods [27]. Briefly, each mouse was carefully maintained under light anesthesia by inhalation of a mixture of 2% isoflurane and oxygen via a nose cone. Left ventricular systolic function was assessed by fractional shortening (FS) and ejection fraction (EF) parameters. Peak early-diastolic transmitral velocities (E wave) and peak late-diastolic transmitral velocities (A wave) across mitral valve inflow were examined on Doppler flow tracings and were used to calculate E/A ratios, a commonly used parameter of left ventricular diastolic function. All echocardiography procedures were performed by the same qualified technician, and the data were averaged from at least three cardiac cycles.

Measurements of cardiac TNF- α , IL-1 β , and NO

The mice were sacrificed under euthanasia at 1 and 4 h after injection of LPS or normal saline. Hearts were harvested and homogenized thoroughly on ice in a lysis buffer. Heart tissue homogenates

were centrifuged at 3,600 g for 10 min, and the supernatant was obtained and stored at -80°C until analysis. TNF- α and IL-1 β protein levels were detected by enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (R&D Systems, Minneapolis, USA). Supernatant protein concentrations were determined using a bicinchoninic acid protein assay kit (Beyotime, Nantong, China); TNF- α and IL-1 β levels are expressed as picograms per milligram of protein. NO production was estimated from the determination of NO oxidation products (nitrate and nitrite), referred to as NOx. NOx levels were determined using Griess reagent.

Western blot analysis of NF- κ B α inhibition (I κ B) and phosphorylated I κ B

The mouse hearts were removed 0.5 h after LPS or normal saline injection and washed once with cold normal saline, then homogenized thoroughly on ice in a split buffer RIPA (Beyotime, Nantong, China) that included phosphatase inhibitor, protease inhibitor, and phenyl methane sulfonyl fluoride. The homogenate was centrifuged for 30 min at 12,000 rpm and 4°C. Protein concentrations of the homogenates were determined using a bicinchoninic acid protein assay kit (Beyotime, Nantong, China). The lysates (100 mg of protein) were separated via 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a PVDF membrane. The membrane was incubated in Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBST) supplemented with 5% bovine serum albumin for 1 h to block nonspecific protein binding at room temperature. The membrane was cut according to the marker and then incubated overnight at 4°C in a 1:2,000 dilution of I κ B antibodies or a 1:600 dilution of the phosphorylated I κ B (phospho-I κ B) antibodies (Abcam, Cambridge, UK). After washing the membrane three times with 0.1% TBST, the membrane was incubated in a 1:800 dilution of horseradish peroxidase-conjugated secondary monoclonal antibodies (R&D Systems, Minneapolis, USA) for 1 h at room temperature, washed three times with 0.1% TBST, and then developed using an enhanced chemiluminescence detection reagent (Pierce Biotechnology, Rockford, USA). The results were quantified by scanning densitometry.

Measurements of myocardial lipid peroxidation and superoxide dismutase activity

Heart homogenates were prepared at 12 h after injection of LPS or saline. An assay of thiobarbituric acid - reactive substances (TBARSs) was performed to assess the level of myocardial lipid peroxidation, a marker of oxidative injury. Myocardial superoxide dismutase (SOD) activity was measured using a xanthine oxidase kit (Jiancheng, Nanjing, China). Enzyme activity was presented as the amount of activity units per milligram of protein.

Terminal deoxynucleotidyl transferase-mediated dUTPnick end-labeling assay

Myocardial apoptosis was assessed at 12 h after LPS administration by quantifying the terminal deoxynucleotidyl transferase-mediated dUTPnickend-labeling (TUNEL) assay using apoptosis detection kit in situ (Roche, New Jersey, USA). Mouse hearts were fixed in 10% phosphate-buffered formaldehyde and were paraffin embedded. Sections were incubated with proteinase K for 30 min at room temperature and then washed with PBS. Sections were incubated with the TUNEL reaction mixture and two negative controls for 60 min at 37°C in a humidified atmosphere in the dark, after which 50 μ L of converter-POD was added to the sample at 37°C for 30 min in a humidified chamber. Visualization of the reaction was achieved using a DAB substrate system. Apoptotic cells were counted in five randomly selected fields per section, corresponding to at least 70 cells examined at 40 x magnification. The apoptotic index was expressed as the percentage of the total number of myocardial cells.

Caspase 3/7 activity determination

Cardiac caspase 3/7 activity was monitored by measuring the relative cleavage of Asp-Glu-Val-Asp (DEVD) (Promega, WI, USA). Mouse hearts were harvested at 2 h after LPS or normal saline injection and thoroughly homogenized with 1 mL normal saline on ice. The homogenate was centrifuged at 3,600 g at 4°C for 10 min. Then, Apo-ONE caspase 3/7 reagent (100 μ L) was added to each well in a 96-well plate containing 100 μ L of the sample and incubated at 37°C for 90 min. The resulting fluorescence was determined at 499 nm using a spectrofluorometer.

Statistical analysis

All data are expressed as the means $\pm$ SEMs. Experimental groups were compared using one-way ANOVA followed by the Student-Newman-Keuls test or an unpaired Student's t test. The difference between means was considered statistically significant when P was less than 0.05.

Results

And prevents LPS-induced myocardial malfunction in vivo

Left ventricular function was examined by echocardiography 12 h after vehicle or LPS injection. As shown in Fig. 1 and 2, LPS injection caused significant reduction of left ventricular systolic function in mice. In contrast, And significantly prevented this LPS-induced decrease in the left ventricular EF and FS, indicating that And attenuates LPS-induced myocardial systolic malfunction. In addition, administration of And alone did not alter left ventricular EF and FS compared with control.

To assess the left ventricular diastolic function, E/A ratios were calculated from Doppler-derived mitral inflow measurements. LPS injection reduced E/A ratio values, an effect that was reversed by treatment with And (Fig. 3). These results indicate that And also inhibits LPS-induced diastolic malfunction. No significant differences in E/A ratios were observed between the control and And alone group.

LPS-induced cardiac TNF- α , IL-1 β and NO productions are inhibited by And

Enhanced production of TNF- α , IL-1 β and NO has implicated them as the major mediators of myocardial malfunction during sepsis [4-8]. Therefore, we have investigated the effects of And on LPS-induced cardiac TNF- α , IL-1 β and NO production to examine the anti-inflammatory properties of And. As shown in Fig. 4, TNF- α and IL-1 β concentrations in heart tissue at 4 h after LPS injection was significantly lower in the And+ LPS group than in the LPS group, indicating that And inhibited LPS-induced IL-1 β and TNF- α over-expression in these hearts. Cardiac TNF- α and IL-1 β were not detectable in the control and And alone groups. In addition, LPS increased cardiac NO levels at 12 h after LPS injection, an effect that was reversed by And (Fig. 4).

Fig. 1. Representative M-mode echocardiograms obtained from mice 12 h after injection. (A-D) represent control, LPS, And + LPS, and And alone groups, respectively.

Fig. 2. Pretreatment with And attenuated LPS-induced left ventricular systolic dysfunction, $n = 10$. * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the LPS group.

Fig. 3. Pretreatment with And attenuated LPS-induced left ventricular diastolic dysfunction. Representative transmitral Doppler images for control (A), LPS (B), and And + LPS (C) groups. The E wave represents peak early-diastolic transmitral velocity, and the A wave indicates peak late-diastolic transmitral velocity. (D) shows changes in the E/A ratio, a marker of diastolic function, in mice from various groups, $n = 10$. * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the LPS group.

And does not affect cardiac lipid peroxidation and superoxide dismutase activity

Oxidative damage plays a vital role in endotoxemia, which enhances the production of reactive oxygen species (ROS) and lipid peroxidation (such as TBARS) in hearts, and antioxidants decrease cardiac TBARS levels and improve rats' survival after LPS injection [28]. In addition, And was found to affect ROS production [29]. Therefore, we further observed the effects of And on cardiac TBARS content and SOD activity in mice. As shown in Tables 1 and 2, LPS did not induce an increase in TBARS content and SOD activity in hearts, and And did not alter TBARS content and SOD activity in hearts at 12 h after LPS or saline injection.

And inhibits I κ B phosphorylation induced by LPS in the mouse heart

I κ B phosphorylation is a vital transcription factor for inflammatory gene expression. Moreover, cardiac I κ B over-expression not only inhibits inflammatory gene production but also prevents cardiac malfunction after LPS injection [30]. Total left-ventricular protein was

Fig. 4. Effects of And on LPS-induced cardiac TNF- α , IL-1 β , and NO production in mice. Cardiac TNF- α and IL-1 β productions were examined at 1 and 4 h after injection, and NO production was determined at 12 h after normal saline or LPS injection, $n = 7$. * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the LPS group.

Table 1. TBARS levels in the mouse heart at 12 h after injection

Group	n	TBARS level, nM malondialdehyde/mg protein
Control	10	3.56 $\pm$ 0.23
LPS	10	3.91 $\pm$ 0.56
LPS+And	10	3.75 $\pm$ 0.42
And	10	3.52 $\pm$ 0.35

Table 2. Activity of SOD in the heart of mice at 12 h after injection

Group	n	SOD activity, U/mg protein
Control	10	7.56 $\pm$ 0.51
LPS	10	8.21 $\pm$ 0.67
LPS+And	10	7.65 $\pm$ 0.82
And	10	7.46 $\pm$ 0.68

collected at 0.5 h after injection, and expressions of total I κ B and phospho-I κ B were assessed by Western blotting. Our results suggested that And affects cardiac I κ B phosphorylation in mice, indicating that And inhibited the activation of I κ B. As shown in Fig. 5A, LPS treatment *in vivo* induced an increase in cardiac I κ B phosphorylation. Meanwhile, And significantly reversed LPS-induced phosphorylation of I κ B in hearts.

Fig. 5. Effects of And on LPS-induced I κ B phosphorylation in the mouse heart. The upper row shows representative Western blot analysis of total and phospho-I κ B in the mouse heart. The lower row represents image analysis of total and phospho-I κ B, n = 5. *P < 0.05 compared with the control group; #P < 0.05 compared with LPS group.

Fig. 6. And inhibited LPS-induced cardiomyocyte apoptosis and cardiac caspase 3/7 activation. (A-D) represent photomicrographs of TUNEL-stained sections from the control, LPS, And + LPS, and And alone groups, respectively. (E) Quantification of TUNEL-positive cardiomyocytes at 12 h after normal saline or LPS injection. (F) Cardiac caspase 3/7 activity examined at 2 h after injection, n = 10. *P < 0.05 compared with the control group; #P < 0.05 compared with the LPS group.

And inhibits LPS-induced cardiac apoptosis and cardiac caspase 3/7 activation

LPS induced a rapid activation of apoptosis to affect myocardial malfunction [31]. To examine whether the cardio protective effects of And are related to the prevention of LPS-induced cardiac apoptosis, cardiac sections were examined by TUNEL staining for apoptosis. Fewer apoptotic cells were found in the control group (Fig. 6A), compared with the LPS

group at 12 h after LPS injection (Fig. 6B). In contrast, And significantly suppressed LPS-induced myocardial apoptosis (Fig. 6C). Moreover, caspase 3/7 activities in hearts from the LPS group were significantly elevated at 2 h after LPS injection compared with the control group, and caspase 3/7 activities in hearts at 2 h after LPS injection was lower in the And + LPS group than in the LPS group (Fig. 6D).

Discussion

The results of the present study have demonstrated that And reversed LPS-induced cardiac malfunction in mice by inhibiting cardiac apoptosis, cardiac I κ B phosphorylation, and TNF- α , IL-1 β , and NO production. Our study found that And significantly reversed LPS-induced reduction of left ventricular EF, FS, and E/A ratio values *in vivo*, suggesting that And attenuates cardiac systolic and diastolic malfunction in endotoxin-induced sepsis.

The mechanisms of LPS-induced cardiac malfunction remain controversial. Various inflammatory mediators, such as TNF- α and IL-1 β , support a vital role in the development of myocardial malfunction in sepsis [4-6]. LPS, TNF- α and IL-1 β have direct negative inotropic effects on cardiomyocytes via induction of NO synthase. And over-production of NO causes contractile malfunction via cyclic guanosine 3', 5'-monophosphate in sepsis [8, 32, 33]. Moreover, phosphorylation of I κ B has been shown to lead to the expression of NF- κ B-controlled genes for cytokines that are associated with cardiac malfunction in the adult mammalian heart [34].

In the study reported herein, LPS administration led to phosphorylation of I κ B and over-production of TNF- α , IL-1 β , and NO in hearts. And inhibited I κ B phosphorylation and the production of TNF- α , IL-1 β and NO in mice hearts after LPS injection. These observations indicated that And suppressed I κ B phosphorylation as well as the production of TNF- α , IL-1 β and NO *in vivo*, resulting in the reversal of cardiac malfunction in LPS-induced mice.

On the other hand, some studies have suggested that the generation of ROS, such as superoxide and peroxynitrite, plays a vital role in the induction of myocardial malfunction caused by LPS [35]. We further examined myocardial SOD activity and TBARS content in mice and found that LPS did not increase myocardial SOD activity and TBARS content at 12 h after injection, which is consistent with a previous study [36]. Moreover, And did not affect myocardial TBARS levels and SOD activities in LPS-induced mice. These results indicate that the protections of And against LPS-induced cardiac malfunction do not prevent oxidative stress. However, although myocardial SOD activity and TBARS content did not increase at 12 h after LPS treatment, the possibility that oxidative stress could play a role in LPS-induced cardiac malfunction cannot be ruled out. For instance, different results could be obtained for measurements made earlier or later than 12 h after LPS injection [37].

Because it is well known that LPS-induced activation of apoptotic pathways results in cardiac malfunction [7, 38], we further evaluated whether And prevented LPS-induced cardiac apoptosis and found that And significantly inhibited cardiac caspase 3/7 activation and apoptosis induced by LPS. Further studies will be needed to determine the potential mechanisms for inhibition of LPS-induced cardiomyocyte apoptosis by And; such studies should consider the opening of the mitochondrial permeability transition pore [39], stimulation of calcium-sensing receptors [40], and activation of cannabinoid type-2 receptors as possible mechanisms [41].

In summary, our study is the first to demonstrate that And prevents LPS-induced cardiac malfunction in mice by inhibiting cardiac myocardial apoptosis and I κ B phosphorylation. These findings indicate that And may be a potential agent for confronting septic cardiac malfunction.

Disclosure Statement

We declare that there are no conflicts of interest in this study.

References

- Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR: Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001;29:1303-1310.
- Becker JU, Theodosis C, Jacob ST, Wira CR, Groce NE: Surviving sepsis in low-income and middle-income countries: new directions for care and research. *Lancet Infect Dis* 2009;9:577-582.
- Parrillo JE, Parker MM, Natanson C, Suffredini AF, Danner RL, Cunnion RE, Ognibene FP: Septic shock in humans. Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. *Ann Intern Med* 1990;113:227-242.
- Niu J, Azfer A, Kolattukudy PE: Protection against lipopolysaccharide-induced myocardial dysfunction in mice by cardiac-specific expression of soluble Fas. *J Mol Cell Cardiol* 2008;44:160-169.
- Sharma AC: Sepsis-induced myocardial dysfunction. *Shock* 2007;28:265-269.
- Rudiger A, Singer M: Mechanisms of sepsis-induced cardiac dysfunction. *Crit Care Med* 2007;35:1599-1608.
- Wang X, Zingarelli B, O'Connor M, Zhang P, Adeyemo A, Kranias EG, Wang Y, Fan GC: Overexpression of Hsp20 prevents endotoxin-induced myocardial dysfunction and apoptosis via inhibition of NF-kappaB activation. *J Mol Cell Cardiol* 2009;47:382-390.
- Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL: Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 1992;257:387-389.
- Parker MM, McCarthy KE, Ognibene FP, Parrillo JE: Right ventricular dysfunction and dilatation, similar to left ventricular changes, characterize the cardiac depression of septic shock in humans. *Chest* 1990;97:126-131.
- Merx MW, Weber C: Sepsis and the heart. *Circulation* 2007;116:793-802.
- Xia YF, Ye BQ, Li YD, Wang JG, He XJ, Lin X, Yao X, Ma D, Slungaard A, Hebbel RP, Key NS, Geng JG: Andrographolide attenuates inflammation by inhibition of NF-kappa B activation through covalent modification of reduced cysteine 62 of p50. *J Immunol* 2004;173:4207-4217.
- Aromdee C: Andrographolide: progression in its modifications and applications - a patent review (2012 - 2014). *Expert Opin Ther Pat* 2014;24:1129-1138.
- Mishra SK, Tripathi S, Shukla A, Oh SH, Kim HM: Andrographolide and analogues in cancer prevention. *Front Biosci (Elite Ed)* 2015;7:255-266.
- Lu WJ, Lin KH, Hsu MJ, Chou DS, Hsiao G, Sheu JR: 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* 2012;84:914-924.
- Awang K, Abdullah NH, Hadi AH, Fong YS: Cardiovascular activity of labdane diterpenes from *Andrographis paniculata* in isolated rat hearts. *J Biomed Biotechnol* 2012;2012:876458.
- Wang YJ, Wang JT, Fan QX, Geng JG: Andrographolide inhibits NF-kappaBeta activation and attenuates neointimal hyperplasia in arterial restenosis. *Cell Res* 2007;17:933-941.
- Woo AY, Waye MM, Tsui SK, Yeung ST, Cheng CH: Andrographolide up-regulates cellular-reduced glutathione level and protects cardiomyocytes against hypoxia/reoxygenation injury. *J Pharmacol Exp Ther* 2008;325:226-235.
- Zhu T, Wang D, Zhang W, Liao X, Guan X, Bo H, Sun J, Huang N, He J, Zhang Y, Tong J, Li C: Andrographolide Protects against LPS-Induced Acute Lung Injury by Inactivation of NF-kB. *PLoS ONE* 2013;8:e56407.
- Peng T, Hu M, Wu TT, Zhang C, Chen Z, Huang S, Zhou XH: Andrographolide suppresses proliferation of nasopharyngeal carcinoma cells via attenuating NF-kappaB pathway. *Biomed Res Int* 2015;2015:735056.
- Hua Z, Frohlich KM, Zhang Y, Feng X, Zhang J, Shen L: Andrographolide inhibits intracellular Chlamydia trachomatis multiplication and reduces secretion of proinflammatory mediators produced by human epithelial cells. *Pathog Dis* 2015;73:1-11.
- Li Y, Yan H, Zhang Z, Zhang G, Sun Y, Yu P, Wang Y, Xu L: Andrographolide derivative AL-1 improves insulin resistance through down-regulation of NF-kappaB signalling pathway. *Br J Pharmacol* 2015;172:3151-3158.
- Yu Z, Lu B, Sheng Y, Zhou L, Ji L, Wang Z: Andrographolide ameliorates diabetic retinopathy by inhibiting retinal angiogenesis and inflammation. *Biochim Biophys Acta* 2015;1850:824-831.

- 23 Nguyen VS, Loh XY, Wijaya H, Wang J, Lin Q, Lam Y, Wong WS, Mok YK: Specificity and inhibitory mechanism of andrographolide and its analogues as antiasthma agents on NF-kappaB p50. *J Nat Prod* 2015;78:208-217.
- 24 Zhai Z, Qu X, Li H, Ouyang Z, Yan W, Liu G, Liu X, Fan Q, Tang T, Dai K, Qin A: Inhibition of MDA-MB-231 breast cancer cell migration and invasion activity by andrographolide via suppression of nuclear factor-kappaB-dependent matrix metalloproteinase-9 expression. *Mol Med Rep* 2015;11:1139-1145.
- 25 Zhang QQ, Zhou DL, Ding Y, Liu HY, Lei Y, Fang HY, Gu QL, He XD, Qi CL, Yang Y, Lan T, Li JC, Gong P, Wu XY, Yang X, Li WD, Wang LJ: Andrographolide inhibits melanoma tumor growth by inactivating the TLR4/NF-kappaB signaling pathway. *Melanoma Res* 2014;24:545-555.
- 26 Karin M, Yamamoto Y, Wang QM: The IKK NF-kappa B system: a treasure trove for drug development. *Nat Rev Drug Discov* 2004;3:17-26.
- 27 Semeniuk LM, Kryski AJ, Severson DL: Echocardiographic assessment of cardiac function in diabetic db/db and transgenic db/db-hGLUT4 mice. *Am J Physiol Heart Circ Physiol* 2002;283:H976-H982.
- 28 Ben-Shaul V, Lomnitski L, Nyska A, Zurovsky Y, Bergman M, Grossman S: The effect of natural antioxidants, NAO and apocynin, on oxidative stress in the rat heart following LPS challenge. *Toxicol Lett* 2001;123:1-10.
- 29 Shen YC, Chen CF, Chiou WF: Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone andrographolide. *Planta Med* 2000;66:314-317.
- 30 Haudek SB, Spencer E, Bryant DD, White DJ, Maass D, Horton JW, Chen ZJ, Giroir BP: Overexpression of cardiac I-kappaBalpha prevents endotoxin-induced myocardial dysfunction. *Am J Physiol Heart Circ Physiol* 2001;280:H962-H968.
- 31 Lancel S, Joulin O, Favory R, Goossens JF, Kluza J, Chopin C, Formstecher P, Marchetti P, Neviere R: Ventricular myocyte caspases are directly responsible for endotoxin-induced cardiac dysfunction. *Circulation* 2005;111:2596-2604.
- 32 Stein B, Frank P, Schmitz W, Scholz H, Thoenes M: Endotoxin and cytokines induce direct cardiodepressive effects in mammalian cardiomyocytes via induction of nitric oxide synthase. *J Mol Cell Cardiol* 1996;28:1631-1639.
- 33 Tatsumi T, Akashi K, Keira N, Matoba S, Mano A, Shiraishi J, Yamanaka S, Kobara M, Hibino N, Hosokawa S, Asayama J, Fushiki S, Fliss H, Nakagawa M, Matsubara H: Cytokine-induced nitric oxide inhibits mitochondrial energy production and induces myocardial dysfunction in endotoxin-treated rat hearts. *J Mol Cell Cardiol* 2004;37:775-784.
- 34 Haudek SB, Spencer E, Bryant DD, White DJ, Maass D, Horton JW, Chen ZJ, Giroir BP: Overexpression of cardiac I-kappaBalpha prevents endotoxin-induced myocardial dysfunction. *Am J Physiol Heart Circ Physiol* 2001;280:H962-H968.
- 35 Ferdinandy P, Danial H, Ambrus I, Rothery RA, Schulz R: Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure. *Circ Res* 2000;87:241-247.
- 36 Jozefowicz E, Brisson H, Rozenberg S, Mebazaa A, Gele P, Callebert J, Lebuffe G, Vallet B, Bordet R, Tavernier B: Activation of peroxisome proliferator-activated receptor-alpha by fenofibrate prevents myocardial dysfunction during endotoxemia in rats. *Crit Care Med* 2007;35:856-863.
- 37 Khodir AE, Ghoneim HA, Rahim MA, Suddek GM: Montelukast attenuates lipopolysaccharide-induced cardiac injury in rats. *Hum Exp Toxicol* DOI: 10.1177/0960327115591372.
- 38 Lancel S, Joulin O, Favory R, Goossens JF, Kluza J, Chopin C, Formstecher P, Marchetti P, Neviere R: Ventricular myocyte caspases are directly responsible for endotoxin-induced cardiac dysfunction. *Circulation* 2005;111:2596-2604.
- 39 Li D, Liu M, Tao TQ, Song DD, Liu XH, Shi DZ: Panax quinquefolium saponin attenuates cardiomyocyte apoptosis and opening of the mitochondrial permeability transition pore in a rat model of ischemia/reperfusion. *Cell Physiol Biochem* 2014;34:1413-1426.
- 40 Lu FH, Fu SB, Leng X, Zhang X, Dong S, Zhao YJ, Ren H, Li H, Zhong X, Xu CQ, Zhang WH: Role of the calcium-sensing receptor in cardiomyocyte apoptosis via the sarcoplasmic reticulum and mitochondrial death pathway in cardiac hypertrophy and heart failure. *Cell Physiol Biochem* 2013;31:728-743.
- 41 Li Q, Wang F, Zhang YM, Zhou JJ, Zhang Y: Activation of cannabinoid type 2 receptor by JWH133 protects heart against ischemia/reperfusion-induced apoptosis. *Cell Physiol Biochem* 2013;31:693-702.