Sinomenine prevents the development of cardiomyopathy in diabetic rats by inhibiting inflammatory responses and blocking activation of NF-κB

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Abstract. Diabetic cardiomyopathy is a severe complication of diabetes mellitus (DM). The goal of current work was to study the effects of sinomenine on streptozotocin-induced cardiomyopathy in rats. DM in rats was induced by intraperitoneal injection of streptozotocin. Cardiac function was evaluated by measuring left ventricle end-diastolic diameter, left ventricle end-systolic diameter and ejection fraction. Cardiac inflammation was evaluated by the degree of infiltration of T lymphocytes and the levels of pro-inflammatory cytokines. Sinomenine attenuated diabetic symptoms without affecting plasma glucose. Cardiac dysfunction in the sinomenine-treated diabetic rats was significantly improved, as reflected by decreased levels of left ventricle end-diastolic diameter, left ventricle end systolic diameter and an increased level of ejection fraction. Sinomenine observably reduced cardiomyocyte hypertrophy in DM rats. Moreover, sinomenine reduced infiltration of CD3^{+} and CD68^{+} positive cells and decreased the levels of tumor necrosis factor-α, interlukin-1 and interlukin-6. Finally, sinomenine-treated rats showed a reduced expression of NF-κB and an increased expression of IκB in the myocardium compared with the myocardium of untreated diabetic rats. Our results indicate sinomenine significantly improves cardiac function in diabetic rats, which may be attributed to the deactivation of NF-κB and the blockade of inflammatory cytokine-mediated immune reactions.

Key words: Sinomenine — Diabetic cardiomyopathy — CD3^{+} — CD68^{+} — NF-κB

Introduction

Diabetic cardiomyopathy (DCM) is a severe complication of diabetes mellitus (DM) and is characterized by cardiac dysfunction and abnormal myocardial structure. DCM is independent of coronary artery disease and systemic hypertension (Sowers et al. 2001). As many lines of evidence (Zannad et al. 1999; From et al. 2006) have described previously, the prevalence of heart failure in DM patients varies from 12% to 48%, and the incidence of DM patients with non-ischemic advanced heart failure is 19.7% (From et al. 2006). Notably, more than 70% of DM patients die from cardiovascular disease, and this ratio is 2–3-fold higher than the mortality by cardiovascular disease in non-diabetic patients (Boudina and Abel 2010; Zhang et al. 2012). A better understanding of the pathophysiology is a prerequisite for the development of new medicines. It is

well-accepted that DCM is a multifactor illness, which involves the activation of the rennin-angiotensin system (RAS), oxidative stress, cardiac inflammation, cardiomyocyte apoptosis and interstitial fibrosis, all of which are activated by hyperglycemia (Boudina and Abel 2010; Zhang et al. 2012). In recent years, many studies have noted that cardiac inflammation may be core element of DCM. In DCM patients, hyperglycemia and dyslipidemia correlate with increased expression and secretion of cytokines. In turn, the levels of cytokines, such as tumor necrosis factor-a (TNF-α) and interleukin-6 (IL-6), are closely associated with left ventricular diastolic dysfunction (Dint et al. 2009; Mahmoud and Al-Ozairi 2013). Furthermore, inflammation can signal to other pathways through cross talk that can also contribute to the formation of DCM *via* induction of oxidative stress and the activation of RAS. Etanercept (a well known TNF-α inhibitor) attenuates myocardial ischemia/reperfusion injury by decreasing the inflammatory response and oxidative stress, while TNF-α itself induces apoptosis in rat cardiomyocytes and upregulates the angiotensin II type 1 receptor on cardiac fibroblasts (Gurantz et al. 2005; Yang et al.

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2014; Yu et al. 2014). In summary, these results suggest that active compounds with anti-inflammatory properties may be useful in preventing DCM.

Sinomenine (Sin, $C_{19}H_{23}NO_4$, m.w. 329 D, Fig. 1) is an active alkaloid extracted from the root of the climbing plant *Sinomenium acutum*, known popularly as Chinese moonseed. It was firstly identified by Ishiwari in 1920 (Mayeda 1953). As reported previously, the compound possesses a diversified set of bioactivities, including anti-arrhythmic, antianginal, analgesic, anti-inflammatory and sedative activities (Zhao et al. 2012). In *vivo* studies, sinomenine effectively reduced the inflammatory response in animal models of arthritis and uveitis (Mu et al. 2013; Song et al. 2013). In an *in vitro* study, sinomenine blockaded activation of immune cells (T lymphocytes, dendritic cells, macrophages) and reduced production of pro-inflammatory cytokines (TNF-α, IL-1) (Wang and Li 2011; Tong et al. 2015). Notably, a recent report demonstrated that sinomenine suppresses TNF-α, IL-1 and IL-6 release in a dose-dependent fashion, and simultaneously reduces nuclear translocation of nuclear transcription factor-kappa B (NF-κB) in AGEs-activated retinal microglia in culture (Wang et al. 2007). Consequently, on the basis of these lines of evidence, we hypothesized that sinomenine may exert protective effects in DCM by suppressing myocardial inflammation and its downstream signaling targets.

Materials and Methods

Animal experiments and treatment

Ninety Wistar rats (180–220 g, male, six weeks old) were obtained from the animal department of Zhejiang University (Hangzhou, China). The animals were housed and allowed free access to a standard diet and tap water. The study was approved by the Animal Ethics Committee of Fenghua Peo-
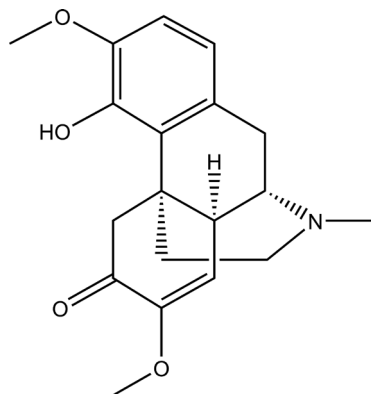
Figure 1. Chemical structure of sinomenine.

ples Hospital (Fenghua, China). All the animal experiments were carried out according to the U.K. animals (Scientific Procedures) Act, 1986 and associated guidelines.

Diabetic rats were induced by a single *intraperitoneal* (*i.p.*) injection of streptozotocin (STZ, 60 mg/kg, Sigma, St. Louis, MO, USA) in fresh 0.1 M citrate buffer (pH 4.5, immediately used after preparation), while normal rats were injected with an equal volume of citrate buffer. The preparation of 0.1 M citrate buffer was as following: Solution A was produced by dissolving 2.1 g citric acid into 100 ml distilled water, and Solution B was formed by dissolving 2.94 g sodium citrate into 100 ml distilled water; 0.1 M citrate buffer was prepared by the mixture of solution A and solution B with a ratio of 1:1.32. Five days later, the STZ-treated rats ($n = 80$) with blood glucose levels ≥16.7 mM were classified as being diabetic. Subsequently, these diabetic rats were randomly divided into five groups as follows: DM group, vehicle-treated rats; Sin30, Sin60, Sin120 groups, diabetic rats treated with sinomenine at doses of 30, 60 and 120 mg/kg; Los group, diabetic rats treated with losartan at dose of 30 mg/kg (sinomenine purity >97%, losartan purity >99%; Aladdin biological technology co., LTD, Shanghai, China). The appropriate vehicle or drug was administered orally to all the rats at the same time every day for ten weeks, following the induction of DM. The total incidence of induced DM in the rats was 81.25% (65/80), and during the experimental period, the mortality of the DM rats was 36.92% (41/65), which breaks down as follows: DM group (seven rats), Sin30 (seven rats), Sin60 (nine rats), Sin120 (ten rats), Los group (eight rats). The normal control group contained ten rats.

Measurement of cardiac function

Ten weeks after drug administration, echocardiography was performed to evaluate the cardiac function of the diabetic rats. Briefly, rats were anesthetized with pentobarbital (*i.p.*, 50 mg/kg). Left ventricle end-diastolic diameter (LVEDD), left ventricle end systolic diameter (LVESD), diastolic inter-ventricular septal wall thickness (IVSD), ejection fraction (EF) and heart rate (HR) were recorded using an echocardiography machine equipped with a 12-MHz linear probe. The parameters were measured at least three times and then averaged.

The experimental animals were euthanized by CO_2 inhalation, and then the hearts were removed and weighed. Heart weight (HW) and the ratio of HW to body weight (BW) were detected. Finally, the left ventricle was quickly cut into three parts. The first part was immediately stored at –80°C for analysis of protein, the second part was used to measure production of pro-inflammatory cytokines, and the last part was fixed in 4% paraformaldehyde for histopathological and immunohistochemical study.

Detection of plasma glucose

After evaluation of cardiac function, blood samples were drawn from the abdominal artery, and the concentrations of glucose were measured using a glucometer (Changsha Sinocare Inc. China).

Histopathological and immunohistochemical studies

The tissue was fixed in 4% paraformaldehyde and embedded in paraffin, and 5-μm sections were stained with hematoxylin-eosin. Pathological scores were recorded based on the cardiomyocyte hypertrophy in each slice (Yu et al. 2012). Cardiomyocyte hypertrophy was evaluated by the measurement of cells size, which is usually represented by the short-axis diameters. The short-axis diameters were determined by the observation and measurement of the shortest diameters that passed through the cell nucleus. Short-axis diameters of cardiomyocytes were assayed for 10 myocytes *per* section at 400-fold magnification, using an inverted microscope (Olympus, Tokyo, Japan) equipped with an ocular micrometer, and then were averaged based on the data from 10 myocytes. For the study of immunohistochemistry (Wen et al. 2013), the sections were incubated with anti-CD68^{+} (Abcam, Cambridge, MA, USA) or anti-CD3^{+} (Abcam, Cambridge, MA, USA) antibodies overnight, and then treated with the corresponding secondary antibody for 30 min. Ultimately, a DAB kit (Vectorlabs, USA) was used to color the sections, and the immunohistochemical staining was quantified by Image Pro Plus on 10 fields for the left ventricle.

Measurement of pro-inflammatory cytokines in cardiac tissue

For detection of inflammatory cytokines, the cardiac tissue was cut into pieces and then homogenized and lysed. After centrifugation at $16000 \times g$ for 10 min (4°C), the supernatants was collected and stored at −80°C for further analysis. The levels of TNF-α, IL-1 and IL-6 in supernatants were determined by commercial ELISA kits (Invitrogen, Camarillo, CA).

Western blot analysis

After rinsing with PBS, heart tissue was treated with lysis buffer. Following a centrifugation step ($12000 \times g$, 15 min), the supernatant was removed and stored at −80°C for protein analysis. Subsequently, 50 μg of extracted protein was loaded and separated by SDS-PAGE followed by electrophoretic transfer to nitrocellulose filters. The membranes were blocked with 5% non-fat dry milk and incubated with different primary antibodies at 4°C overnight. The following antibodies were used in this study: IκB and NF-κB p65 (Abcam, Cambridge, MA, USA). After incubation with the secondary antibody, the blots were visualized using an ECL kit (Pierce Biosciences, Billerica, MA, USA).

Statistical analysis

The results are expressed as the means ± SD. SPSS (version 14.0) software was used to perform the statistical analysis. One-way analysis of variance (ANOVA) and Dunnett's t-test were used to assess the significance of any change between groups. A value of $p < 0.05$ was considered to be statistically significant.

Results

Sinomenine reduces STZ-induced diabetic pathology without affecting plasma glucose

In the DM group, STZ-induced diabetic rats exhibited markedly lower body weight and higher blood glucose level compared with the negative control (NC) group ($p < 0.01$; Fig. 2). However, administration of sinomenine to diabetic
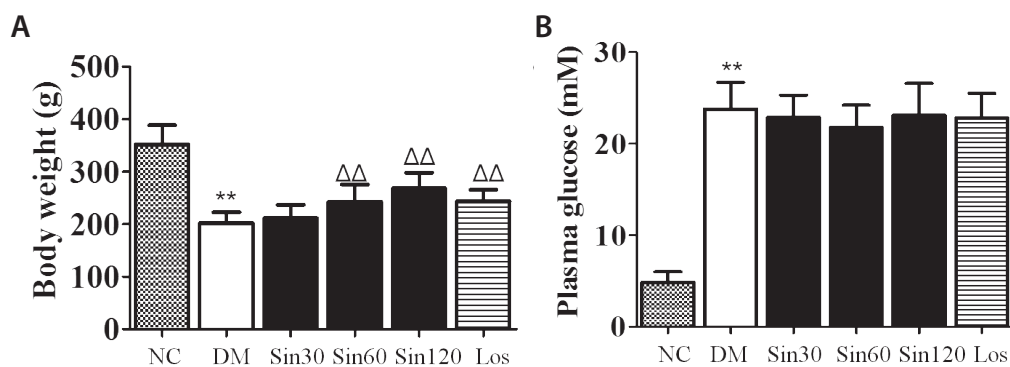
Figure 2. Effect of sinomenine on diabetic performance in experimental diabetic rats. At the end of study, the body weights of the mice were measured (**A**) and the blood samples were collected from abdominal artery for the plasma glucose detection (**B**). The data are presented as the mean ± SD, $n = 7–10$; [**] $p < 0.01$ *vs.* NC group; [ΔΔ] $p < 0.01$ *vs.* DM group. NC, normal control group; DM, group of rats with induced diabetes mellitus; Sin30, Sin60, Sin120, diabetic rats treated with sinomenine at doses of 30, 60 and 120 mg/kg; Los, diabetic rats treated with losartan at dose of 30 mg/kg.

rats strikingly increased their body weights compared with the DM group ($p < 0.01$). In addition, sinomenine had no effect on the level of blood glucose. We also found that Los-treated diabetic rats showed similar results to those observed with sinomenine.

Sinomenine prevents diabetic-induced cardiac dysfunction

As shown in Table 1 and Figure 3H, in the DM group, STZ-induced diabetic rats exhibited significant cardiac dysfunction, as demonstrated by increased values of IVSD, LVEDD, LVESD, cardiac index, and decreased values of heart rate and EF ($p < 0.01$). However, oral administration of sinomenine to the diabetic rats caused decreases in IVSD, LVEDD, LVESD and cardiac index and increases in the heart rate and EF in comparison to the DM group ($p < 0.05$). These findings suggest sinomenine could improve diabetic induced-cardiac dysfunction.

Sinomenine improves cardiac histopathology in diabetic rats

To evaluate the cardioprotective effects of sinomenine further, myocardial structural was analyzed by H&E staining. Perinuclear vacuolization occurred in the DM rats (Fig. 3B) but this property was normalized in sinomenine-treated rats (Fig. 3D,E). Additionally, cell size was measured to evaluate the cardiomyocyte hypertrophy of the left ventricle. As can be seen in Fig. 3G, cardiomyocyte diameters in the DM group were obviously greater than those in the normal group ($p < 0.01$). Notably, in sinomenine-treated diabetic rats, cardiomyocyte hypertrophy was clearly reduced compared with those in the DM group ($p < 0.05$). These results indicate definitively that sinomenine can ameliorate cardiac histopathology in diabetic rats.

Sinomenine inhibits cardiac inflammation in STZ-induced diabetic rats

Previous studies (Yu et al. 2012; Wen et al. 2013) demonstrated that impaired cardiac function is closely associated with cardiac inflammatory responses. To further disclose the potential mechanism of sinomenine action, we focused on the effects of sinomenine on cardiac infiltration of T lymphocytes and macrophages and on the production of pro-inflammatory cytokines in diabetic rats. As seen in Figure 4, in the DM group, the numbers of CD3^{+} (T lymphocyte, Fig. 4B) and CD68^{+} positive cells (macrophage, Fig. 4E) were significantly higher than those in the normal group ($p < 0.01$). However, sinomenine markedly reduced infiltration of these cells in diabetic rats ($p < 0.01$). Moreover, the levels of TNF-a, IL-1 and IL-6 in diabetic myocardium were reduced significantly after oral administration of sinomenine (Fig. 5; $p < 0.01$). These results revealed that sinomenine could reduce cardiac inflammation in STZ-induced diabetic rats.

Sinomenine prevents the activation of NF-κB in diabetic rats

It is well-known that cross-talk between pathways can occur through NF-κB, which is a key point of intersection of several cytokine-mediated pathways that are involved in amplification of the inflammatory response (Lorenzo et al. 2011). To uncover possible mechanisms by which sinomenine inhibits cardiac inflammation, we measured cardiac expression of NF-κB and IκB in the various groups. Figure 6 presents the decreased expression of IκB and increased expression of NF-κB in the diabetic rats, indicative of activated NF-κB signaling; this is consistent with the result reported previously (Wang et al. 2005). Strikingly, sinomenine abolished cardiac activation of NF-κB in the diabetic rats ($p < 0.01$).

Table 1. Sin alleviated STZ induced left ventricular dysfunction in diabetic rats

Group	Heart rate (bpm)	IVSD (mm)	LVEDD (mm)	LVESD (mm)	EF (%)
NC	376 ± 14	1.37 ± 0.09	2.82 ± 0.17	1.50 ± 0.08	84.6 ± 2.2
DM	$264 \pm 9^{**}$	$1.97 \pm 0.14^{**}$	$3.40 \pm 0.28^{**}$	$2.27 \pm 0.16^{**}$	$71.4 \pm 3.9^{**}$
Sin30	$288 \pm 13^{\triangle}$	1.84 ± 0.13	3.31 ± 0.22	2.12 ± 0.13	74.8 ± 3.1
Sin60	$306 \pm 17^{\triangle\triangle}$	$1.72 \pm 0.12^{\triangle\triangle}$	$3.17 \pm 0.19^{\triangle}$	$1.98 \pm 0.19^{\triangle}$	$76.2 \pm 3.3^{\triangle\triangle}$
Sin120	$325 \pm 15^{\triangle\triangle}$	$1.55 \pm 0.16^{\triangle\triangle}$	$3.01 \pm 0.20^{\triangle\triangle}$	$1.77 \pm 0.22^{\triangle\triangle}$	$82.3 \pm 3.5^{\triangle\triangle}$
Los	$338 \pm 19^{\triangle\triangle}$	$1.40 \pm 0.11^{\triangle\triangle}$	$3.02 \pm 0.13^{\triangle\triangle}$	$1.80 \pm 0.12^{\triangle\triangle}$	$80.4 \pm 2.4^{\triangle\triangle}$

Data are presented as means ± SD, $n = 7$–10. [**] $p < 0.01$ *vs.* NC group; [△] $p < 0.05$, [△△] $p < 0.01$ *vs.* DM group. IVSD, diastolic interventricular septal wall thickness; LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end systolic diameter; EF, ejection fraction = [(LVEDD3- LVESD3)/LVEDD3] × 100%; NC, normal control group; DM, group of rats with induced diabetes mellitus; Sin30, Sin60, Sin120, diabetic rats treated with sinomenine at doses of 30, 60 and 120 mg/kg; Los, diabetic rats treated with losartan at dose of 30 mg/kg.
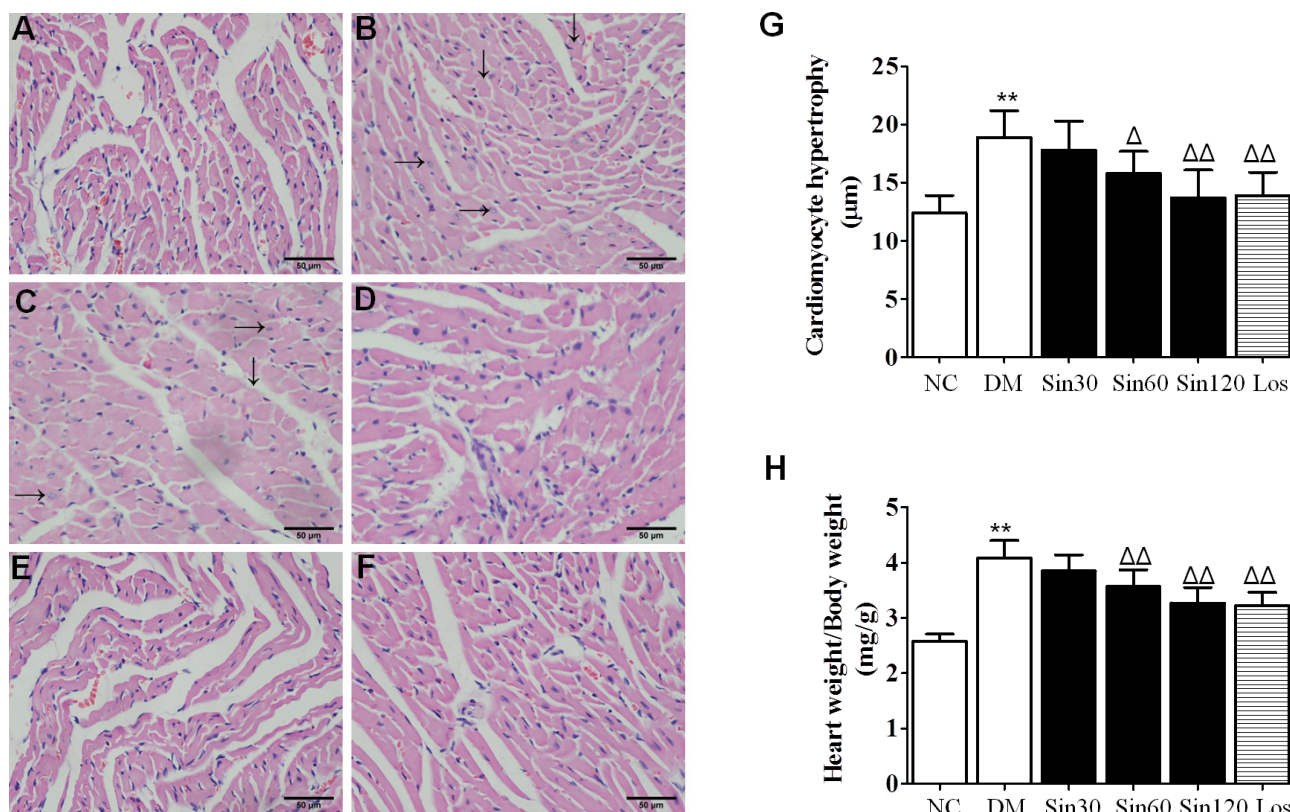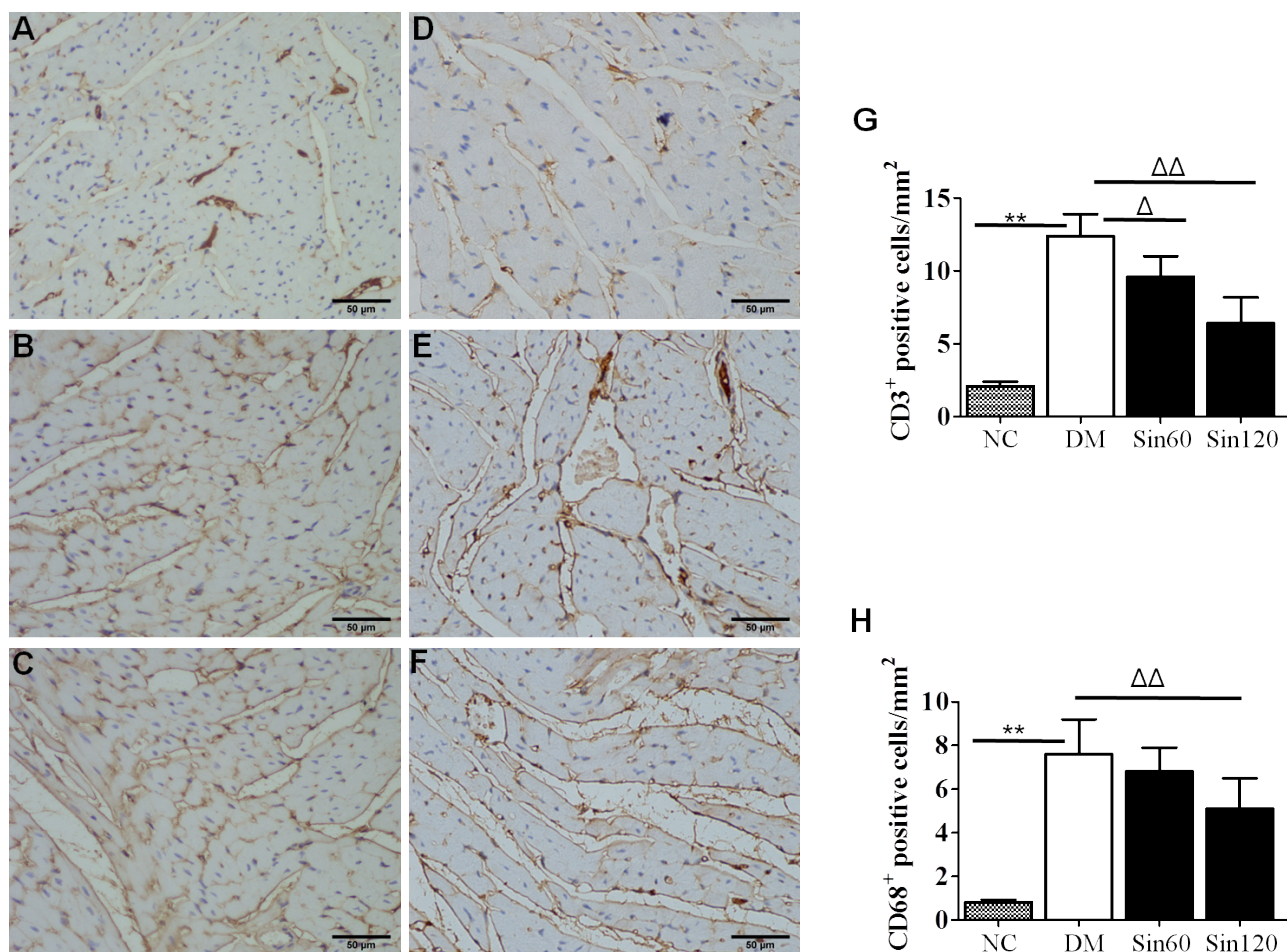
Figure 3. Effect of sinomenine on cardiac histopathology in experimental diabetic rats. **A.** Cardiac tissue of normal rats. **B.** Cardiac tissue in DM rats exhibits significant perinuclear vacuolization (horizontal arrow) and cardiomyocyte hypertrophy (vertical arrow). Cardiac tissue in Sin30 (**C**), Sin60 (**D**), Sin120 (**E**), and Los (**F**) groups. The indicated scales in the pictures are 50 μm. **G.** Quantitative analysis of cardiomyocyte diameter. **H.** Cardiac index as determined by the value of heart weight/body weight. Data are presented as the mean ± SD, $n = 7$–10; [**] $p < 0.01$ *vs.* NC group; [Δ] $p < 0.05$, [ΔΔ] $p < 0.01$ *vs.* DM group. (For abbreviations, see Fig. 1).

Discussion

Many reports have confirmed that myocardial inflammation is the main contributor to DCM. In diabetic patients, increased production of inflammatory cytokines is associated with diastolic dysfunction (Hoffman et al. 2013), and severe, reversible cardiomyopathy is associated with systemic inflammatory response syndrome in the setting of diabetic, hyperglycemic, non-ketotic syndrome (Berk et al. 2015). Moreover, hyperglycemia-induced myocardial damage is mediated both by pro-inflammatory cytokines and by macrophage migration inhibitory factor (Yu et al. 2011). In diabetic animal models, many reports have confirmed the enhanced infiltrations of T lymphocytes and macrophages, and the increased production of pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) in the myocardium (Yu et al. 2012; Wen et al. 2013). It is widely believed that myocardial inflammation in DCM is originally induced by hyperglycemia (Pan et al. 2014). Subsequently, inflammatory factors maybe disrupt cardiac function directly as demonstrated by the following observations. High levels of IL-1, IL-6 and TNF-α directly inhibit rat cardiomyocyte contractility in *vitro* (Hobai et al. 2015). TNF-α provokes a concentric cardiac hypertrophic phenotype, induces apoptosis of cardiac myocytes, and contributes to progressive left ventricular wall thinning and adverse cardiac remodeling (Bozkurt et al. 1998; Dibbs et al. 2003; Haudek et al. 2007). Mice with cardiac-specific over-expression of IL-1 show concentric left ventricular hypertrophy with preserved left ventricular systolic function. IL-1 also induces programmed cell death in cultured cardiac myocytes through NOS induction (Ing et al. 1999; Nishikawa et al. 2006). Regarding IL-6, continuous over-expression of both IL-6 and the IL-6 receptor (IL-6R) in double transgenic mice is associated with cardiac myocyte hypertrophy. However, neither IL-6 nor IL-6R over-expression alone is sufficient to induce detectable myocardial abnormalities due to the low expression level of IL-6R in cardiac myocytes (Saito et al. 1992; Hirota et al. 1995).

It is well-known that cytokines mediate cardiac inflammation via downstream activation of NF-κB. Under physi-
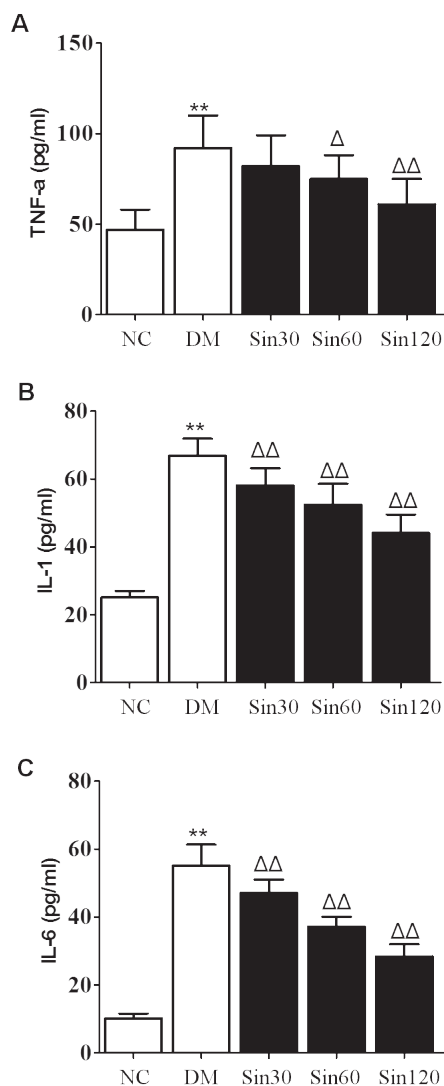
Figure 4. Effect of sinomenine on inflammatory cells' infiltration in cardiac tissue of diabetic rats. **A.** CD3^{+} positive cells in normal rats. **B.** CD3^{+} positive cells in DM rats. **C.** CD3^{+} positive cells in Sin 120 group. **D.** CD68^{+} positive cells in normal rats. **E.** CD68^{+} positive cells in DM rats. **F.** D68^{+} positive cells in Sin120 group. The indicated scales in the pictures are 50 μm. **G.** Quantitative analysis of CD3^{+} positive cells numbers. **H.** Quantitative analysis of CD68^{+} positive cells numbers. Data are presented as the mean ± SD, $n = 7$–10; [**] $p < 0.01$ *vs.* NC group; [Δ] $p < 0.05$, [ΔΔ] $p < 0.01$ *vs.* DM group. (For abbreviations, see Fig. 1).

ological conditions, NF-κB is bound to its inhibitory proteins, known as IκBs (Lorenzo et al. 2011). When the cell receives a pathological stimulus, such as high glucose, oxidative stress or production of pro-inflammatory cytokines, NF-κB will be activated by dissociation from IκB. Subsequently, the activated form of NF-κB will translocate into the nucleus, bind to NF-κB-consensus sequences in genomic DNA (named κB sites) and thereby regulate gene transcription (Lorenzo et al. 2011). Of note, the expression levels of almost 200 target genes have been reported to be modulated by NF-κB, and most of them are involved in the modulation of inflammatory responses (Hall et al. 2006; Lorenzo et al. 2011). Interestingly, activation of NF-κB induces transcription of inflammatory cytokines, and the released cytokines can bind with their receptors, which results in further activation of NF-κB signaling. As reported previously, when cardiomyocytes are treated with a TNF-α antagonist (etanercept), they shows decreased levels of NF-κB signaling (Xu et al. 2008), and in TNF-α receptor deficient mice, NF-κB activation is greatly suppressed when compared to wild-type mice (Aoki et al. 2014). Therefore, overactivation of inflammatory cytokines and of NF-κB results in a vicious spiral instead of a classical negative feedback.
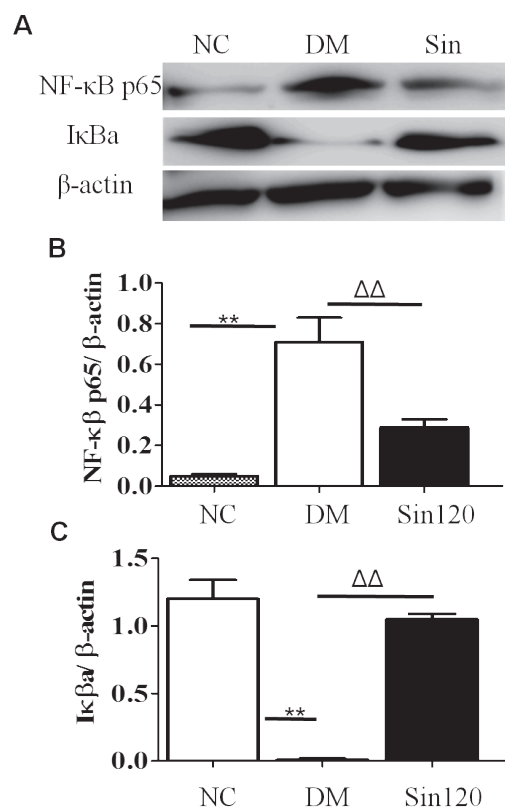
The role of NF-κB in DCM is well-established. As discussed previously, in patients with myocarditis and dilated cardiomyopathy, activation of NF-κB closely correlates with left ventricular function (Alter et al. 2006). In animal models, cardiomyocyte over-expression of IκB (inhibitor of NF-κB) attenuated TNF-α-induced fractional shortening and ventricular hypertrophy (Higuchi et al. 2006), and blockade of NF-κB activation improved cardiac function and survival in male TNF-transgenic mice (Kawamura et al. 2005).

Figure 5. Effect of sinomenine on pro-inflammatory cytokines levels in cardiac tissue of diabetic rats. **A.** TNF-α. **B.** IL-1. **C.** IL-6. Data are presented as the mean ± SD, $n = 5$; $^{**} p < 0.01$ *vs.* NC group; $^{\triangle} p < 0.05$, $^{\triangle\triangle} p < 0.01$ *vs.* DM group. IL-1, interlukin-1; TNF-α, tumor necrosis factor-α. (Other abbreviations, see Fig. 1).

Figure 6. Effect of sinomenine on NF-κB signaling in diabetic rats. **A.** Representative lanes of targeted proteins. **B.** Western blot analysis of NF-κB p65. **C.** Western blot analysis of IκB. Data are presented as the mean ± SD, $n = 5$; $^{**} p < 0.01$ *vs.* NC group; $^{\triangle\triangle} p < 0.01$ *vs.* DM group. (For abbreviations, see Fig. 1).

Therefore, it is proposed that inhibition of NF-κB may be a useful strategy for preventing DCM, especially when pro-inflammatory cytokines are overactivated.

Although the role of pro-inflammatory cytokines is clear, the results of large multicenter trials of etanercept in moderate to severe heart failure did not demonstrate any clinical benefits and may adversely affect the course of the disease (Anker and Coats 2002; Coletta et al. 2002). It is widely believed that the disordered cytokines networks in DCM are complex, and strict control of one or several cytokines is insufficient to inhibit the myocardial lesions. Sinomenine,

first discovered in 1920, has been confirmed to possess anti-inflammatory activity. In the past 30 years, the therapeutic efficacy and lower side effects of purified sinomenine in patients with rheumatoid arthritis and nephritis have been confirmed in open clinical trials (Cheng et al. 2009; Wang and Li 2011). In recent years, the anti-inflammatory mechanism of sinomenine has been uncovered. At the cellular level, sinomenine reduces proliferation of lymphocytes, keeps T lymphocyte subtypes balanced, reduces the invasion and migration ability of monocytes, prevents DC maturity and inhibits DC antigen presentation (Zhao et al. 2007; Cheng et al. 2009; Tong 2015). At the molecular level, sinomenine attenuates nuclear translocation of the NF-κB p65 subunit and the DNA-binding activity of NF-κB (Wang et al. 2007; Cheng et al. 2009; Zhao et al. 2013). In addition, sinomenine also reduces the production of IL-1β and TNF-α in synovial cells of rats with adjuvant-induced arthritis (Anker and Coats 2002; Zhao et al. 2012; Mu et al. 2013; Zhao et al. 2013).

In this work, it is firstly discovered that treatment of sinomenine significantly improves cardiac function in diabetic rats, as evidenced by the increases in the heart rate and

EF, and by decreased values of IVSD, LVEDD and LVESD. Of note, when comparing the Los group and the Sin120 group, it seems that losartan is more effective in improving heart rate and the IVSD, whereas sinomenine is more effective in improving LVEDD, LVESD and EF. However, after careful analysis, we confirmed that there were no significant differences in the cardioprotective effects between the groups treated with losartan or sinemonine. Therefore, it can be concluded that Sin120 and Los groups possess the same pharmacologic effects in the prevention of ventricular dysfunction. Interestingly, sinomenine did not alter plasma glucose, which suggests the cardiovascular protective effects are independent of anti-diabetic activity. Moreover, it is worth mentioning that a portion of DCM patients have symptomatic features, such as hypotension, and losartan may not be a suitable drug for these patients (Bellmann and Tschöpe 2014; Dimitropoulos et al. 2014). Furthermore, angiotensin receptor blockers easily damage renal function in some special classes of patients, including the elderly, children, and heart failure and renal artery stenosis patients (Maillard et al. 2001; Kiykim et al. 2004). Therefore, for these patients, sinomenine may be provided as an alternative medication, based on its safety and efficacy. Finally, further research showed that sinomenine observably inhibited infiltration of T lymphocytes and macrophages, reduced the levels of pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) and blocked cardiac activation of NF-κB. Our results showed that sinomenine therapy can undermine cardiac inflammatory responses and deactivate the NF-κB signaling pathway.

Conclusions

Taken together, the findings of the present study demonstrate that sinomenine improves cardiac function in DM rats. Sinomenine preserves the myocardium by reducing NF-κB activation and inhibiting the production of pro-inflammatory cytokines that mediate myocardial inflammation.

Conflict of interest. All authors have approved the final article, and declare that they have no conflict of interest.

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