

# Daidzein attenuates abdominal aortic aneurysm through NF- $\kappa$ B, p38MAPK and TGF- $\beta$ 1 pathways

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**Abstract.** The current study focuses on the protection of daidzein on nerves, as daidzein was demonstrated to have a protective effect on neurons of the central nervous system in a glutamate excitotoxicity and oxygen/glucose deprivation model. However, the effect of daidzein on the abdominal aortic aneurysm (AAA) remains unclear. The angiotensin II-induced AAA mouse model was utilized in the present study to determine the effect of daidzein on AAA. The results demonstrated that daidzein significantly attenuated incidence of AAA, max aortic aneurysm and mortality in the angiotensin II-induced AAA mice. Daidzein had an anti-inflammatory effect by inhibiting tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin 1 $\beta$  (IL-1 $\beta$ ) and nuclear factor  $\kappa$ B (NF- $\kappa$ B) protein expression. In addition, daidzein strongly suppressed the gene expression of cyclooxygenase (COX)-2, matrix metalloproteinase 2 (MMP-2), tissue inhibitor of metalloproteinase 1 (TIMP-1), transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), and inhibited inducible nitric oxide synthase (iNOS) protein expression in angiotensin II-induced AAA mice. It also inhibited phosphorylation of the p38 mitogen-activated protein kinase (MAPK) signaling pathway. These results demonstrate, to the best of our knowledge for the first time, that the anti-inflammatory effects and inhibitory mechanism of daidzein attenuates AAA in angiotensin II-induced mice. Daidzein contains strong anti-inflammatory activity and affects various mechanism pathways including the NF- $\kappa$ B, p38MAPK and TGF- $\beta$ 1 pathway.

## Introduction

Abdominal aortic aneurysm (AAA) is a degenerative disease characterized by structural damage to the wall of the abdominal aorta and the gradual expansion into a pulsating mass (1). It is

generally accepted that AAA is the expansion of the abdominal aortic artery diameter to greater than 1.5 times the neighboring normal artery (2). To the best of our knowledge, an epidemiological survey remains to be conducted in China thus far. In western countries, the incidence of AAA is increasing; a previous study reported incidence of 12% in male hypertensive patients between the ages of 60 and 74 and 20-29% in male siblings of the patients (3). The natural rupture rate of two years for symptomatic AAA without treatment is up to 50%. The overall AAA mortality rate has been reported to be 80-90% (4). Therefore, the aim of the present study is to investigate the pathogenesis of AAA to aid the development of early drug treatment programs.

The interaction of various factors, including genetic, environmental and biochemical factors, is the etiology of AAA (5). Human AAA is predominantly a result of hypertension, atherosclerosis and other diseases and conditions, including congenital aortic hypoplasia, syphilis, trauma, Takayasu arteritis, Marfan syndrome, infection and Bechet syndrome (6). Previous studies demonstrated that the pathological process of the aneurysm derives from the abnormal degradation of the artery wall, and this alteration is often present in atherosclerotic lesions (7-9). These studies suggest that atherosclerotic plaques may weaken the arterial wall structure or lead to a significant reduction in the mechanical strength of the abdominal aorta, resulting in focal protrusion to form a tumor, as the lipid infiltration directly destroys the normal structure of the arterial wall and the compression of the plaque results in the reduction of blood flow, thus causing ischemia in the film (10). Numerous clinical studies have demonstrated that there is widespread inflammation in the majority of AAA walls (11-13).

There are 12 types of isoflavones in soy, divided into three categories, daidzin, genistin and glycitin (14). The soy isoflavones exist in free form, glucoside, acetyl-glucoside or malonyl-glucoside (14). Daidzein has been reported to exhibit anti-oxidative and anti-inflammatory activity, reduce estrogenic activity, and prevent menopause, osteoporosis and cardiovascular diseases (15). Thus, this may suggest that daidzein attenuates AAA, and that the NF- $\kappa$ B, p38MAPK and TGF- $\beta$ 1 pathways may be critical in the action of daidzein against AAA.

## Materials and methods

**Reagents.** Angiotensin II and daidzein were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tumor necrosis factor  $\alpha$

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(TNF- $\alpha$ ) and interleukin 1 $\beta$  (IL-1 $\beta$ ) mouse enzyme-linked immunosorbent assay (ELISA) antibody set was obtained from eBioscience Inc. (San Diego, CA, USA). Bradford reagent and SDS-PAGE were obtained from Beyotime Institute of Biotechnology (Jiangsu, China).

**Angiotensin II-induced AAA mice model and treatment.** Male BALB/C male mice (n=30; age, 8 weeks; weight, 20–22 g) were purchased from the Experimental Center of The First Affiliated Hospital of Dalian Medical University (Liaoning, China). This is a prospective interventional animal study. All mice were housed at 22 $\pm$ 2°C with relative humidity of 55 $\pm$ 5% and a 12-h dark:light cycle. All mice were fed with normal chow *ad libitum*, housed in a pathogen-free barrier facility and bred as littermate controls. Mice were randomly divided into three groups (n=10) as follows: Control, mice were infused subcutaneously (i.s.) with saline vehicle for 4 weeks as previously described (16); angiotensin II-treated, mice were i.s. with 1,000 ng/kg/min angiotensin II, then received an intraperitoneal injection (i.p.) of saline vehicle for 4 weeks as previously described (17); daidzein, angiotensin II-induced mice were treated with 0.2 mg/kg daidzein i.p. for 4 weeks. Mice was sacrificed by an excess of anesthetic (500  $\mu$ l 3% pentobarbital sodium; Sigma-Aldrich)

**Quantification of angiotensin II-induced AAA mice.** The perfusion aorta was fixated with 4% cold paraformaldehyde (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), and exposed under a dissecting microscope (Olympus SZX7; Olympus Corporation, Tokyo, Japan) to remove the periaortic adventitial tissue from the aortic wall. The gross appearances of the aorta were then observed to measure the maximal external diameter of the suprarenal aorta using the MultiGauge 3000 imaging processing software (Fujifilm Holdings Co., Tokyo, Japan). The increase in the aortic outer diameter was defined as >50% and used to describe the development of the aortic aneurysm.

**Measurement of inflammation.** Serum was obtained from the peripheral vessel and centrifuged at 1,200  $\times$  g for 10 min at room temperature. TNF- $\alpha$  and IL-1 $\beta$  serum levels were determined using the eBioscience mouse ELISA antibody set. Absorbance was determined at 450 nm wavelength using an ELISA reader (Spectramax Plus, Molecular Devices, LLC, Sunnyvale, CA, USA).

**Western blot analysis of nuclear factor  $\kappa$ B (NF- $\kappa$ B), inducible nitric oxide synthase (iNOS) and p38 mitogen-activated protein kinase (MAPK).** The whole aorta was harvested and homogenated with phosphatase inhibitor cocktail (Roche Diagnostics, Basel, Switzerland) in radioimmunoprecipitation assay lysis buffer (EMD Millipore, Billerica, MA, USA). The mixed solution was centrifuged at 1,200  $\times$  g for 10 min at 4°C, and protein concentration was determined using Bradford's reagent (Beyotime Institute of Biotechnology, Jiangsu, China) according to the manufacturer's instructions. Equal amounts of protein were subjected to 8–10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Sigma-Aldrich) for 50 min at 60 V and transferred to nitrocellulose membranes (110 V for 75 min;

EMD Millipore). The membranes were blocked with 5% skimmed milk in tris-buffered saline with 0.1% Tween 20 (Beyotime Institute of Biotechnology) for 1 h at 4°C. The membranes were then incubated with rabbit anti-mouse NF- $\kappa$ B (cat no. sc-372; 1:1,000), rabbit anti-mouse iNOS (cat no. sc-650; 1:1,500), rabbit anti-mouse p38MAPK (cat no. sc-101427; 1:500) p-p38MAPK (cat no. sc-7973; 1:500) and  $\beta$ -actin (cat no. sc-130656 1:2,000) primary antibodies at 4°C overnight, all Santa Cruz Biotechnology, Inc., (Dallas, TX, USA). Subsequently, the membranes were incubated with anti-mouse IgG horseradish peroxidase-conjugated secondary antibodies (cat no. 7074, 1:5000; Cell Signaling Technology, Inc., Danvers, MA, USA), and proteins were detected with the SuperSignal West Femto chemiluminescent Substrate (Thermo Fisher Scientific, Inc., Rockford, IL, USA).

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of cyclooxygenase (COX)-2, matrix metalloproteinase 2 (MMP-2), tissue inhibitor of metalloproteinase 1 (TIMP-1) and transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1).** Total RNA was isolated from the aorta samples using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. Total RNA (500 ng) was utilized to synthesize cDNA using TaqMan Gold RT-PCR kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. cDNA (1  $\mu$ g) was used to compound DNA. RT-qPCR was performed using the SYBR green JumpStart Taq ReadyMix (Sigma-Aldrich), SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and the iCycler iQ Real-Time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). RT-qPCR was performed using the SYBR green JumpStart Taq ReadyMix (Sigma-Aldrich), SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and the iCycler iQ Real-Time PCR detection system (Bio-Rad Laboratories, Inc.). The reactions were performed at 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 60°C for 30 sec, 72°C for 45 sec and 4°C for saving. The sequences for gene-specific primers are demonstrated in Table I.

**Statistical analysis.** Data are presented as means  $\pm$  standard error. Statistical analysis was performed using Student's t-test for paired or unpaired data, and data were assessed using SPSS software, version 13 (SPSS, Inc., Chicago, IL, USA).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Daidzein affects angiotensin II-induced AAA mice.** The chemical structure of daidzein is presented in Fig. 1. To evaluate the possible effect of daidzein on AAA mice, AAA was induced by continuous angiotensin II treatment. Mice were treated with normal saline and 0.2 mg/kg daidzein per day. Incidence of AAA, max aortic aneurysm and mortality rate of the angiotensin II-induced AAA mice were observed to be higher compared with those of the control mice (Fig. 2;  $P < 0.01$ ). In addition, administration of daidzein significantly

Table I. Sequences for gene-specific primers.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
COX-2	CACTCAGTTTGTGAGTCATTC	GATTAGTACTGTAGGGTTAATG
MMP-2	ACACTGGGACCTGTCACTCC	TGTCAGTGTCCGCCAAATAA
TIMP-1	GCAACTCGGACCTGGTCATAA	CGGCCCGTGATGAGAAACT
TGF- $\beta$ 1	TGCTTCAGCTCCACAGAGAA	TGGTTGTAGAGGGCAAGGAC
GAPDH	TGTACCGTCTAGCATATCTCCGAC	ATGATGTGCTCTAGCTCTGGGTG

COX-2, cyclooxygenase 2; MMP-2, matrix metalloproteinase 2; TIMP-1, tissue inhibitor of metalloproteinase 1; TGF- $\beta$ 1, transforming growth factor  $\beta$ 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

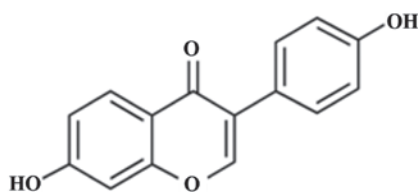


Figure 1. The chemical structure of daidzein.

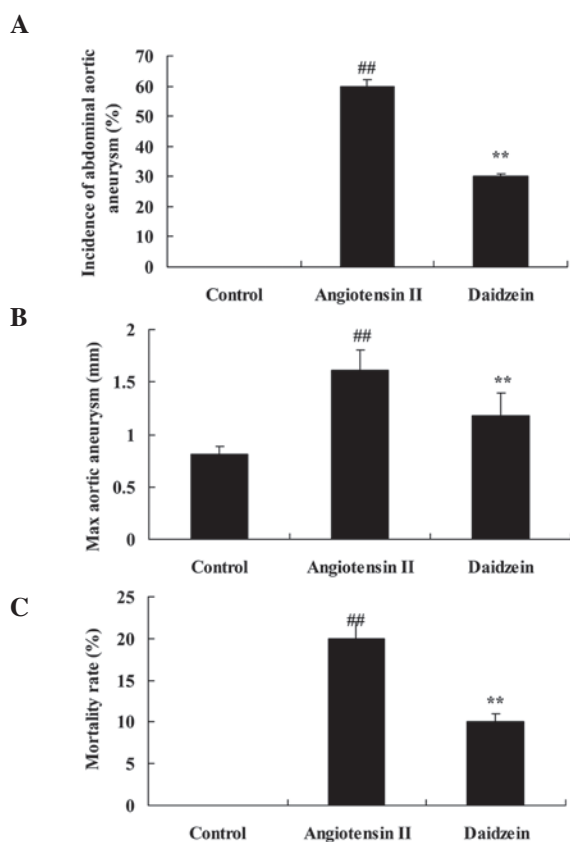


Figure 2. The effect of daidzein on angiotensin II-induced mice. (A) Incidence of abdominal aortic aneurysm, (B) max aortic aneurysm and (C) mortality rate in control, angiotensin II-treated and daidzein-treated mice. ## $P$ <0.01 vs. the control group and \*\* $P$ <0.01 vs. the angiotensin II group. Data are presented as means  $\pm$  standard error of the mean.

attenuated these indexes in the angiotensin II-induced AAA mice compared with the angiotensin II model mice (Fig. 2;  $P$ <0.01).

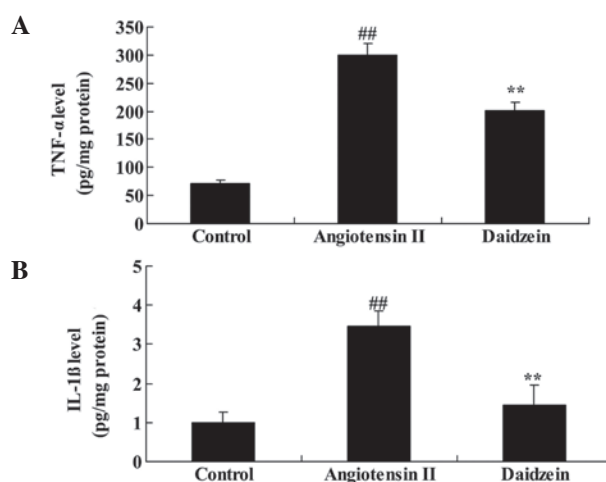


Figure 3. The effect of daidzein on inflammation in angiotensin II-induced mice. (A) TNF- $\alpha$  and (B) IL-1 $\beta$  serum levels in control, angiotensin II-treated and daidzein-treated mice. ## $P$ <0.01 vs. the control group and \*\* $P$ <0.01 vs. the angiotensin II group. Data are presented as means  $\pm$  standard error of the mean. TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; IL-1 $\beta$ , interleukin 1 $\beta$ .

*Daidzein affects inflammation in angiotensin II-induced mice.* To determine the effect of daidzein on inflammation in the angiotensin II-induced mice, TNF- $\alpha$  and IL-1 $\beta$  serum levels were measured. As demonstrated in Fig. 3, angiotensin II infusion significantly increased the serum levels of TNF- $\alpha$  and IL-1 $\beta$  in AAA mice, compared with the control mice ( $P$ <0.01). However, a significant reduction in the TNF- $\alpha$  and IL-1 $\beta$  serum levels was demonstrated in the daidzein-treated group, compared with the angiotensin II model mice (Fig. 3;  $P$ <0.01).

*Daidzein affects NF- $\kappa$ B protein expression in angiotensin II-induced mice.* To confirm the mechanism of daidzein on inflammation in the angiotensin II-induced mice, NF- $\kappa$ B protein expression was assessed. Western blot analysis results demonstrated that angiotensin II significantly increased the NF- $\kappa$ B protein expression in the AAA mice, compared with the control group (Fig. 4;  $P$ <0.01). Additional treatment with daidzein significantly inhibited the activation of NF- $\kappa$ B protein expression in the AAA mice compared with the angiotensin II model mice (Fig. 4;  $P$ <0.01).

*Daidzein affects COX-2 gene expression in angiotensin II-induced mice.* A previous study demonstrated that

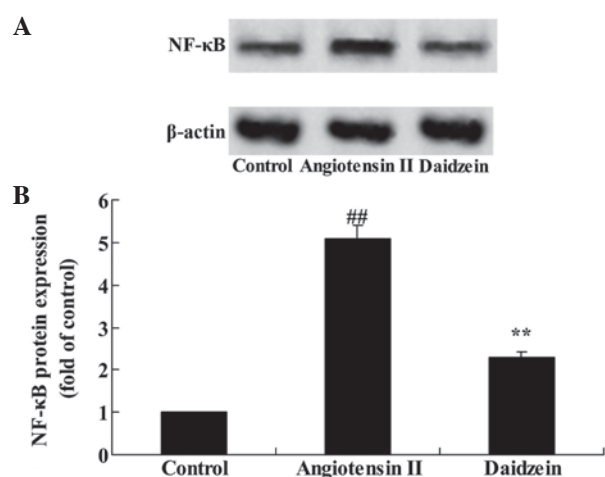


Figure 4. The effect of daidzein on the NF-κB protein expression levels in angiotensin II-induced mice. (A) Western blotting assay and (B) statistical analysis of the NF-κB protein expression in control, angiotensin II-treated and daidzein-treated mice. <sup>##</sup>P<0.01 vs. the control group and <sup>\*\*</sup>P<0.01 vs. the angiotensin II group. Data are presented as means ± standard error of the mean. NF-κB, nuclear factor κB.

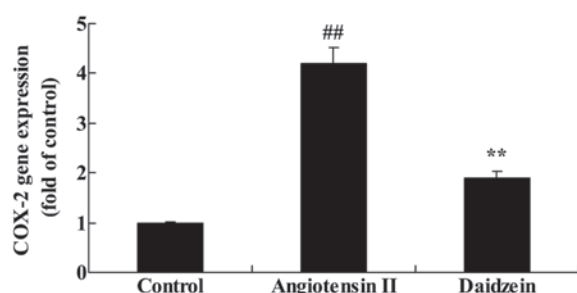


Figure 5. The effect of daidzein on COX-2 gene expression in control, angiotensin II-treated and daidzein-treated mice. <sup>##</sup>P<0.01 vs. the control group and <sup>\*\*</sup>P<0.01 vs. the angiotensin II group. COX-2, cyclooxygenase-2. Data are presented as means ± standard error of the mean.

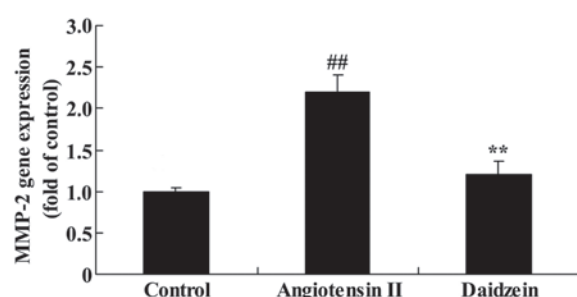


Figure 6. The effect of daidzein on the MMP-2 gene expression in control, angiotensin II-treated and daidzein-treated mice. <sup>##</sup>P<0.01 vs. the control group and <sup>\*\*</sup>P<0.01 vs. the angiotensin II group. Data are presented as means ± standard error of the mean. MMP-2, metalloproteinase 2.

the suppression of COX-2 expression has an effect on angiotensin II-induced AAA mice (18). Therefore, the effect of daidzein on COX-2 gene expression in angiotensin II-induced mice was determined. As demonstrated in Fig. 5, COX-2 gene expression was significantly increased in the angiotensin II-induced AAA mice compared with the control mice (P<0.01). COX-2 gene expression in the daidzein-treated mice

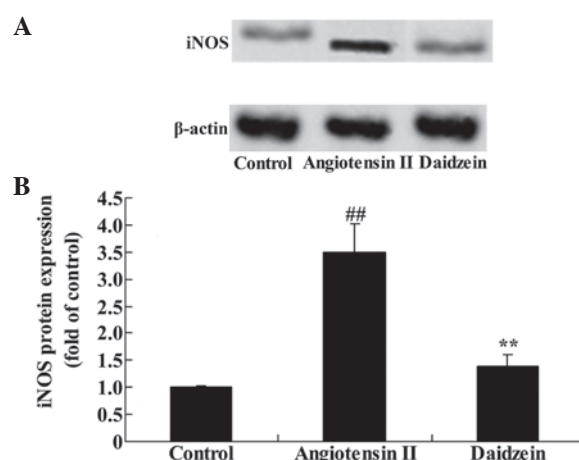


Figure 7. The effect of daidzein on iNOS protein expression levels in angiotensin II-induced mice. (A) Western blotting assay and (B) statistical analysis of iNOS protein expression in control, angiotensin II-treated and daidzein-treated mice. <sup>##</sup>P<0.01 vs. the control group and <sup>\*\*</sup>P<0.01 vs. the angiotensin II group. Data are presented as means ± standard error of the mean. iNOS, inducible nitric oxide synthase.

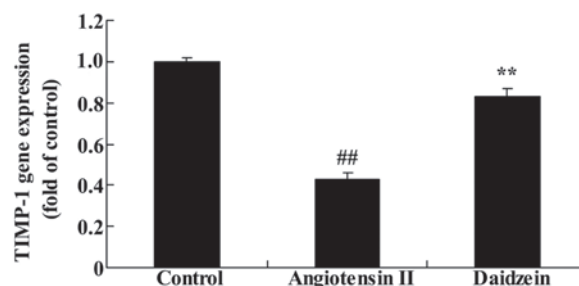


Figure 8. The effect of daidzein on TIMP-1 gene expression in control, angiotensin II-treated and daidzein-treated mice. <sup>##</sup>P<0.01 vs. the control group and <sup>\*\*</sup>P<0.01 vs. the angiotensin II group. Data are presented as means ± standard error of the mean. TIMP-1, tissue inhibitor of metalloproteinase 1.

was significantly suppressed compared with the angiotensin II model mice (Fig. 5; P<0.01).

*Daidzein affects MMP-2 gene expression in angiotensin II-induced mice.* The mediated inflammation in angiotensin II-induced mice was assessed and the MMP-2 gene expression was measured using RT-qPCR analysis. A significant increase was observed in the MMP-2 gene expression in the angiotensin II-induced AAA mice compared with the control mice (Fig. 6; P<0.01). Additional treatment with daidzein significantly suppressed the promotion of MMP-2 gene expression in the angiotensin II-induced AAA mice compared with the angiotensin II model mice (Fig. 6; P<0.01).

*Daidzein affects iNOS protein expression in angiotensin II-induced mice.* Western blot analysis was performed to determine iNOS protein expression levels in the aortic aneurysmal tissue. A significant elevation of iNOS protein expression levels was observed in the angiotensin II-induced AAA mice, compared with the control mice (Fig. 7; P<0.01). Administration of daidzein significantly inhibited the increase of iNOS protein expression compared with the angiotensin II model mice (Fig. 7; P<0.01).



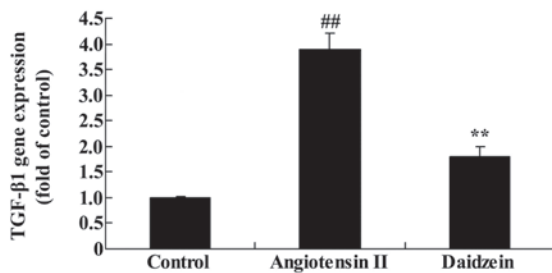


Figure 9. The effect of daidzein on TGF- $\beta$ 1 gene expression in control, angiotensin II-treated and daidzein-treated mice. <sup>##</sup> $P < 0.01$  vs. the control group and <sup>\*\*</sup> $P < 0.01$  vs. the angiotensin II group. Data are presented as means  $\pm$  standard error of the mean. TGF- $\beta$ 1, transforming growth factor  $\beta$ 1.

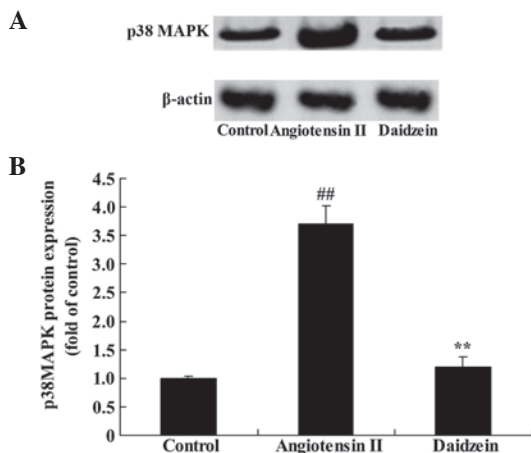


Figure 10. The effect of daidzein on the p38MAPK protein expression levels in angiotensin II-induced mice. (A) Western blotting assay and (B) statistical analysis of the p38MAPK protein expression in control, angiotensin II-treated and daidzein-treated mice. <sup>##</sup> $P < 0.01$  vs. the control group and <sup>\*\*</sup> $P < 0.01$  vs. the angiotensin II group. Data are presented as means  $\pm$  standard error of the mean. MAPK, mitogen-activated protein kinase.

*Daidzein affects TIMP-1 gene expression in angiotensin II-induced mice.* RT-qPCR was utilized to assess the effect of daidzein on TIMP-1 gene expression. As demonstrated in Fig. 8, TIMP-1 gene expression in the angiotensin II-induced AAA mice was lower than that of the control mice ( $P < 0.01$ ). Compared with the angiotensin II model mice, additional treatment with daidzein significantly increased the suppression of TIMP-1 gene expression in the angiotensin II-induced AAA mice (Fig. 8;  $P < 0.01$ ).

*Daidzein affects TGF- $\beta$ 1 gene expression in angiotensin II-induced mice.* RT-qPCR was utilized to assess the effect of daidzein on TGF- $\beta$ 1 gene expression in the angiotensin II-induced mice. As demonstrated in Fig. 9, TGF- $\beta$ 1 gene expression significantly increased in the angiotensin II-induced AAA mice compared with the control mice. However, administration of daidzein significantly inhibited the activation of TGF- $\beta$ 1 gene expression in the angiotensin II-induced AAA mice compared with the angiotensin II model mice (Fig. 9;  $P < 0.01$ ).

*Daidzein affects p38MAPK protein expression in angiotensin II-induced mice.* The expression of p38MAPK protein

was assessed to investigate the role of p38MAPK signaling in the effect of daidzein on angiotensin II-induced AAA mice. Western blot analysis demonstrated that the expression of p38MAPK protein in the angiotensin II-induced AAA mice was significantly higher compared with the control mice (Fig. 10;  $P < 0.01$ ). Following daidzein treatment, the activation of p38MAPK signaling was suppressed in the angiotensin II-induced AAA mice compared with the angiotensin II model mice (Fig. 10;  $P < 0.01$ ).

## Discussion

AAA results from the interaction of various factors, such as anatomical, genetic, environmental and biochemical factors (1). Compared with the structure of suprarenal area of the abdominal aorta, the infrarenal aortic wall is weak and the partial load is higher, short of nourishing blood vessels and without adequate blood supply in cases of AAA (19). The anatomical defect is one of the basic pathological causes of the AAA disease (20). Previous studies demonstrated that immune inflammation is a common mechanism of AAA pathogenesis, which likely promotes the formation of AAA by infiltration of inflammatory cells, inducing inflammatory cytokines and the initiation of apoptosis by the MMP family (20-22). The present study demonstrated that administration of daidzein significantly attenuated these indexes in the angiotensin II-induced AAA mice compared with those of model mice. These results indicate that daidzein may be a novel therapeutic drug for the treatment and prevention of AAA.

Previous studies have demonstrated that inherent inflammation and antigen-induced immune responses during chronic inflammatory response in AAA tissues (21-23). Monocytes accumulate in the outer membrane of the cell to be activated and differentiate into macrophages, an important step for the degradation of the extracellular matrix (5). As a part of a non-specific immune response, lymphocytes may serve a regulatory role in the activity of macrophages, and the accumulation of inflammatory cells is a manifestation of autoimmunity (5). A previous study conducted cellular localization of MMPs by staining anti-MMP-2, -3 and -9 in human AAA specimens, and the results demonstrated that MMP-2 and -9 were located in macrophages, which have strong matrix solubility (24). Previous studies indicated similar results, that MMP-2 and MMP-9 serve important roles in the pathogenesis of AAA, and the positive cells of MMPs are mainly localized in macrophages (25,26). These data suggest that the infiltration of macrophages is vital for the early activity (elastin damage to the arterial wall) of AAA formation (27). As the infiltration degree of inflammatory cells is gradually increased, MMP species are increased with the activity, damage of elastic and collagen fibers, and the dilation extent of the abdominal aorta. The elastin degradation products produced by the degrading extracellular matrix due to the MMPs, may lead to further chemotaxis of mononuclear macrophages that are involved in inflammation (6). The present study demonstrated that daidzein treatment inhibited the promotion of TNF- $\alpha$  and IL-1 $\beta$  serum levels, and activation of NF- $\kappa$ B protein expression in the angiotensin II-induced AAA mice. These results are consistent with Khan *et al* (28), who suggested that daidzein

inhibited the 12-O-tetradecanoylphorbol-13-acetate-induced cutaneous inflammation through the NF- $\kappa$ B and COX-2 expression.

MMPs are predominantly produced by macrophages, T or B lymphocytes, fibroblasts and smooth muscle cells. Macrophages generate MMPs depending on the role of prostaglandin E2 (PGE2), that is synthesized by the arachidonic acid under the effect of a series of enzymes, in which COX-2 is the rate-limiting enzyme. PGE2 inhibits COX-2, thus inhibiting the generation of MMPs (6,29). Previous studies have demonstrated that inflammatory cytokines are associated with the vascular response to injury and atherosclerosis. Numerous inflammatory mediators, such as PGE2, IL-1, IL-6, IL-8, TNF- $\alpha$  and nitric oxide, have direct or indirect angiogenic activity (18,30-32). The results of the present study demonstrated that administration of daidzein significantly suppressed COX-2 and MMP-2 relative gene expression levels, and inhibited iNOS protein expression levels in the angiotensin II-induced AAA mice. Choi *et al* (15) demonstrated that daidzein inhibited inflammation by suppressing iNOS protein expression.

TIMPs are endogenous low-molecular-weight proteins, hydrolytic enzymes in the degradation of the extracellular matrix and endogenous inhibitors of MMPs, serving a role in various pathophysiological processes of the body (33). TIMPs serve a role in cardiac remodeling, myocardial ischemia-reperfusion injury and mediating the remodeling of external cardiac extracellular matrix (34). TIMPs are protease inhibitors secreted by the cells in the body, which may be expressed in the sarcomere of myocardial cells, thin filaments and stromal cells, and may be additionally expressed in tumor cells (35). TIMPs are a family of multi-functional factors, that regulate the activity of MMPs *in vivo*, as TIMP-1 may be bound to numerous MMPs, including MMP-1, 3 and 9 (10). The present study demonstrated that daidzein significantly increased the suppression of the TIMP-1 gene expression in the angiotensin II-induced AAA mice. Soumyakrishnan *et al* (17) indicated that daidzein exhibits an anti-fibrotic effect by reducing the expression of TIMP-1 and TGF- $\beta$ 1 in Bleomycin-induced experimental pulmonary fibrosis. These results demonstrate that the TIMP signaling pathway serves an important role in the effect of daidzein on AAA.

TGF exists widely in the body, regulating cell growth and differentiation. In the cardiovascular system, predominantly TGF- $\beta$ 1 promotes matrix synthesis and secretion (24). Although previous studies suggested that overexpression of TGF- $\beta$ 1 and apoptosis of smooth muscle are increased simultaneously, TGF- $\beta$ 1 has been demonstrated to exhibit different effects in smooth muscle cells. TGF- $\beta$ 1 inhibited apoptosis in cultured bovine aortic smooth muscle cells, however triggered apoptosis in the cultured rat aortic smooth muscle cells (36-38). This effect may be due to the TGF- $\beta$ 1 regulation of the apoptosis of smooth muscle cells dependent on the local microenvironment and the concentration of TGF- $\beta$ 1 (39). In addition, the cytotoxic medium generated by the infiltrated T lymphocytes in the aortic aneurysm results in the apoptosis of smooth muscle cells, thus TGF- $\beta$ 1 may regulate apoptosis by influencing the degree of inflammatory infiltration in the wall of the aneurysm (38,39). Similar to the results of previous

studies, the results of the current study demonstrated that daidzein significantly inhibited the activation of TGF- $\beta$ 1 gene expression in the angiotensin II-induced AAA mice (40,41). The results of the present study demonstrated that the TGF- $\beta$ 1 signaling pathway induced by daidzein serves an important role in the treatment of AAA.

In the p38/MAPK signaling pathway, the phosphokinase p38MAPK mediates the proliferation and migration of vascular smooth muscle cells (VSMCs), and the hypertrophy of VSMCs and the deposition of extracellular matrix may be the required signaling pathway for hypertensive vascular remodeling (42). Protein kinase 1 activated by TGF- $\beta$ 1, combined with protein, may lead to the autophosphorylation of p38MAPK, to activate the p38/MAPK signaling pathway (9). Therefore, TGF- $\beta$ 1 and p38MAPK serve important roles in the occurrence and development of hypertension (9,42). The results of the current study demonstrated that daidzein significantly suppressed the activation of p38MAPK signaling in the angiotensin II-induced AAA mice. Lim *et al* (14) demonstrated that daidzein is a novel inhibitor of protein kinase  $\alpha$  by suppressing the solar ultraviolet-induced MMP-1 through p38. Thus, these data suggest that p38 signaling associates with the effect of daidzein on AAA.

In conclusion, the results of the current study demonstrated that the anti-inflammatory effects and inhibitory mechanism of daidzein attenuate AAA in the angiotensin II-induced mice. The anti-inflammatory effect of daidzein was associated with NF- $\kappa$ B, COX-2, MMP-2 and iNOS expression. In addition, the results suggested that suppression of TIMP, TGF- $\beta$ 1 and p38MAPK signaling promotes the effect of daidzein in AAA. Daidzein had a strong anti-inflammatory activity and affects various mechanism pathways, including NF- $\kappa$ B, p38MAPK and TGF- $\beta$ 1, suggesting it may be a novel therapeutic target for the treatment of AAA formation.

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