Histology and Histopathology

From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE

Astragaloside IV induces endothelial progenitor cell angiogenesis in deep venous thrombosis through inactivation of PI3K/AKT signaling

Xiaojiang Lyu*, Zhigang Yi*, Yun He, Chunfeng Zhang, Ping Zhu and Chonghai Liu

[1]Department of Pediatrics, Affiliated Hospital of North Sichuan Medical College, Nanchong, China

*These authors contributed equally to this work

Summary. Background. Deep vein thrombosis (DVT), referred to as venous thromboembolism, is the third most frequent cardiovascular disease. Endothelial progenitor cells (EPCs) contribute to the recanalization of DVT. Astragaloside IV (AS-IV) has been suggested to have angiogenesis-enhancing effects. Here, we investigate the roles and mechanisms of AS-IV in EPCs and DVT.

Methods. The experimental DVT model was established by inferior vena cava stenosis in rats. EPCs were collected from patients with DVT. Transwell assays were performed to detect cell migration. Tube formation was determined using Matrigel basement membrane matrix and ImageJ software. The thrombus weight and length were measured. Pathological changes were examined by hematoxylin-eosin staining. The production of proinflammatory cytokines was estimated by ELISA. The level of PI3K/AKT-related proteins was measured by western blotting.

Results. AS-IV administration facilitated the migrative and angiogenic functions of human EPCs *in vitro*. Additionally, AS-IV inhibited thrombosis and repressed the infiltration of leukocytes into the thrombus and the production of proinflammatory cytokines in rats. Mechanistically, AS-IV inactivated PI3K/AKT signaling in rats.

Conclusion. AS-IV prevents thrombus in an experimental DVT model by facilitating EPC angiogenesis and decreasing inflammation through inactivation of PI3K/AKT signaling.

Key words: Deep venous thrombosis, Astragaloside IV, Angiogenesis, Leukocytes, Inflammation, PI3K/AKT

Corresponding Author: Chonghai Liu, Affiliated Hospital of North Sichuan Medical College, 1 Maoyuan South Road, Shunqing District, Nanchong, China. e-mail: liuchdoctor@hotmail.com

Introduction

Deep venous thrombosis (DVT) is an obstructive disease with a hindering venous reflux mechanism. It usually involves the lower limb venous system, with clot formation originating in a deep calf vein and propagating proximally (Chen et al., 2022). DVT is a common venous thromboembolic disorder affecting 1.6 per 1000 people annually (Albricker et al., 2022). Post-thrombotic syndrome (PTS), including chronic pain, edema, and leg ulceration, occurs in 43% of patients within two years post-DVT, and 40% of patients presenting with proximal DVT develop a pulmonary embolism. The recurrence of DVT is up to 25% (Waheed et al., 2023). As reported, DVT represents the third most common cause of death from cardiovascular disease after heart attacks and stroke. Even in patients who do not get pulmonary emboli, recurrent thrombosis and PTS are major causes of death (Parker and Thachil 2018; Seifi et al., 2018; Naringrekar et al., 2019). Currently, effective therapeutic options for DVT include anticoagulation, thrombectomy, and pharmacologic thrombolysis (Fuchs et al., 2012). However, these therapeutic methods have the risk of major hemorrhage and wound complications (Kim et al., 2015). Thus, finding novel effective agents for treating DVT is urgent.

Angiogenesis is a critical player in thrombus recanalization (Li and Li, 2016). Endothelial progenitor cells (EPCs) are vascular endothelial precursor cells characterized by their potential to self-renew, migrate, and develop into mature endothelial cells, which have therapeutic efficacies in cardiovascular diseases (Lo Gullo et al., 2018). Accumulating evidence has articulated that recruitment of EPCs to thrombi accelerates thrombus resolution, which is beneficial for DVT treatment (Kong et al., 2016a,b; Li and Li, 2016). Moreover, EPCs are involved in the angiogenesis and repair of injured vasculature (Miller-Kasprzak and Jagodziński, 2007). However, the clinical applications of EPCs face many challenges (Hou and Li, 2018);

therefore, the exploration of the mechanism regulating EPC functions is crucial.

Inflammation plays a critical role in the development of DVT (Liu et al., 2020a). Under pathological conditions, the venous vascular endothelium is activated and recruits leukocytes, which can release pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (Angona et al., 2015). Neutrophils, monocytes, and lymphocytes are three major types of leukocytes and can reflect the inflammation, malnutrition, and immune status of patients (Schrottmaier et al., 2020). The number of neutrophils, monocytes, and lymphocytes increased after DVT onset (Feng et al., 2017). Consequently, a reduction in leukocytes is essential to treat DVT.

Astragaloside IV (AS-IV) isolated from *astragaloside* has been widely used in treating cardiovascular disease in China. Its antioxidant, anti-inflammatory, and anti-apoptotic properties have been well established (Ren et al., 2013; Liu et al., 2020b). AS-IV has been found to suppress the inflammatory response in human bronchial epithelial cells (Hsieh et al., 2022). The water-soluble derivative of AS-IV, astragalosidic acid, shares a similar structure with AS-IV and has a preventive effect on arachidonic acid-induced thrombosis in zebrafish (Wu et al., 2022). Moreover, AS-IV enhances the angiogenic function of adipose-derived mesenchymal stem cells in a hindlimb ischemia model (Wang et al., 2022), accelerates bone regeneration through inducing osteogenesis and angiogenesis (Wang et al., 2021c), and improves angiogenesis in diabetic rats (Wang et al., 2021b). However, the biological functions of AS-IV in DVT remain uncertain.

Collectively, this study was designed to examine the effect of AS-IV on DVT. Phosphoinositide 3-kinase (PI3K)/AKT signaling is involved in the regulation of inflammation, and its activation is associated with thrombosis (Chang et al., 2016). Therefore, this study explores whether the effects of AS-IV on DVT development are mediated by PI3K/AKT signaling. Our findings may contribute to the development of a novel effective treatment for DVT.

Materials and methods

Animals

Sprague-Dawley (SD) rats (male, 200-250 g; SJA Laboratory Animal CO., LTD, Hunan, China) were housed in a specific pathogen-free room with a controlled temperature of 22±2°C and 55±5% humidity under a 12-h light/dark cycle. All rats were given free access to a regular rodent diet. All experiments involving animals were approved by the Animal Ethics Committee of the Affiliated Hospital of North Sichuan Medical College, and all experimental protocols strictly followed the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no.85-23, revised 1996).

Establishment of the experimental DVT model

After one week of acclimation, rats received overnight fasting. Then, the rats were anesthetized with pentobarbital sodium (36 mg/kg; Sigma-Aldrich, Shanghai, China). Subsequently, the rats underwent a midline laparotomy to expose the inferior vena cava (IVC). Thereafter, the IVC was ligated with 7-0 Prolene sutures, and the posterior venous branches were tightened (Yang et al. 2020b). Then, the confluence in the iliac vein was clamped with vascular clips for 15 min. Finally, the incision was sutured, and ceftriaxone sodium (TopScience, Shanghai, China) was used to prevent bacterial infection. The sham operation included the same surgical procedure without IVC stenosis.

Animal groupings

There were a total of 30 rats recruited in this study. Animals were randomly divided into four groups: (a) the sham group, rats received sham operation; (b) the Thrombotic group, rats underwent IVC stenosis; (c) the Thrombotic + DMSO group, rats were administrated 400 μL dimethyl sulfoxide (DMSO; MedChemExpress, Shanghai, China) orally for 14 days after thrombosis induction; (d) the Thrombotic + AS-IV group, AS-IV dry powder (purity: ≥98%) purchased from MedChemExpress was dissolved and diluted with DMSO, and rats were administered 10 mg/kg/day AS-IV in DMSO orally for 14 days after thrombosis induction. A total of five rats died during model establishment (one during operation, two the day after operation, and two three days post-operation). Each group had six rats. The dose of AS-IV was chosen according to a previous study (Qin et al., 2022).

Sample collection

On day 14 post-operation, the animals were intraperitoneally injected with 1% pentobarbital sodium and venous plasma was harvested from the IVC using a vacuum blood tube without anticoagulants. Thereafter, rats were euthanized by decapitation after deep anesthesia. Afterward, after the removal of excessive blood on the thrombi, the IVC containing the thrombus was carefully removed for weight and length assessment. IVCs were split into two parts after clearing unclotted blood: one was fixed with 4% paraformaldehyde for histopathologic analysis and the other was stored at -80°C for western blotting and ELISA.

Histologic analyses

After fixation in 4% paraformaldehyde for 48h, IVCs were dehydrated with graded alcohol, mounted in paraffin, and cut into sections of 4 μm thickness. The sections were then deparaffinized and stained with hematoxylin (Sigma-Aldrich) for 15 min and eosin (Sigma-Aldrich) for 40s. An optical microscope

(Olympus, Tokyo, Japan) was applied to count and sum the neutrophils, monocytes, and lymphocytes from five high-power fields (HPFs).

ELISA

The collected venous plasma was centrifuged at 1600×g for 10 min at 4°C. The serum levels of TNF-α, IL-1β, and IL-6 were assayed with ELISA kits (Multi Sciences, Hangzhou, China). The absorbance value at 450 nm was read via a microplate reader (BioTek, Winooski, VT, USA). The minimum detectable level of the kits for TNF-α, IL-1β, and IL-6 was 0.43, 1.42, and 2.57 pg/mL, respectively.

Western blotting

The frozen IVCs were lysed using RIPA buffer (Sigma-Aldrich) for the isolation of total protein, and the BCA Protein Assay Kit (Innochem, Beijing, China) was utilized to measure the protein concentration. Thereafter, proteins (40 μg) were separated by SDS-PAGE and blotted onto a PVDF membrane. After blocking with 5% skimmed milk for two hours, the membrane was incubated overnight with primary antibodies against phosphorylated PI3K (ab182651, 1:500; Abcam), PI3K (ab191606, 1:1000; Abcam), phosphorylated AKT (ab81283, 1:5000; Abcam), AKT (ab233755, 1:2000; Abcam), GAPDH (ab181603, 1:10000; Abcam) at 4°C, and subsequently incubated with the respective secondary antibodies for 2 h at room temperature. After washing with TBST (Sigma-Aldrich) three times, the bands were visualized by an enhanced chemi-luminescence reagent (Beyotime, Shanghai. China), and the blot intensity was quantified by Image Lab 3.0 software (Bio-Rad, Hercules, CA, USA).

Isolation of EPCs

Ten patients aged 18-60 years with newly diagnosed first idiopathic DVT of the lower limbs at the Affiliated Hospital of North Sichuan Medical College were included in this study. However, patients who had received anticoagulant drugs or undergone surgery prior to blood collection, and patients with a history of diabetes mellitus, hypertension, and other chronic diseases were excluded from this study. The DVT symptoms in enrolled patients lasted 21 days. The protocol was approved by the ethics review committee of the Affiliated Hospital of North Sichuan Medical College. All participants provided their written informed consent. EPCs were isolated as previously described (Zhang et al., 2018). First, peripheral blood (80 mL from each subject) was collected from 10 patients, and peripheral blood mononuclear cells (PBMCs) were isolated through density gradient centrifugation using Histopaque-1077 (Sigma-Aldrich) and then seeded into FN-coated six-well dishes ($2×10^7$ cells/well). Primary cells were incubated with endothelial cell basal medium-

2 (EGM-2; Lonza, Walkersville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich). After four days of incubation, non-adherent cells were removed using phosphate-buffered saline (PBS; Sigma-Aldrich), and new medium was applied. Thereafter, the medium was refreshed every two days. Cells at passage 3 were used for subsequent experiments. To detect the biological functions of AS-IV, EPCs were treated with or without AS-IV (100 μM) (Hsieh et al., 2022).

Transwell detection of cell migration

Cell migration was examined by a Transwell system (Corning, NY, USA). The isolated EPCs were plated into the top compartment containing Matrigel (Sigma-Aldrich), and EBM-2 medium containing 20% FBS was added to the lower reservoir. The cells migrated to the lower surface of the chamber filter after 24h of incubation were fixed and stained with hematoxylin. An optical microscope was used to measure the number of EPCs penetrating the membrane of three random fields.

Tube formation assay

EPCs were seeded into 24-well plates ($5×10^4$ cells/well) coated with Matrigel (80 μL/well) at 37°C. After 24h of incubation, tube-like structures were monitored using an inverted optical microscope (Leica, Wetzlar, Germany) and images were processed using ImageJ software.

Statistics analysis

All experiments were performed with at least three independent repeats. Statistical analysis was analyzed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Data were described as the mean ± standard deviation. One-way analysis of variance followed by Tukey's *post hoc* analysis and Student's t-test were used for comparison analyses. $p<0.05$ was considered statistically significant.

Results

AS-IV facilitates human EPC migration and angiogenesis

Figure 1A shows the chemical structure of AS-IV. Peripheral blood was collected from 10 patients with newly diagnosed DVT to isolate EPCs, and human EPCs at passage 3 were used in this study. The effects of AS-IV on human EPC migration and angiogenesis were detected. As Transwell assays revealed, the AS-IV-treated human EPCs exhibited enhanced migrative capability compared with EPCs without AS-IV treatment (Fig. 1B,C). The angiogenic function of human EPCs was strengthened in the presence of AS-IV as evidenced by increased tube-like structures and tube length (Fig. 1D-F). These results demonstrate that AS-IV enhances

the angiogenic function of human EPCs.

AS-IV inhibits thrombus formation and leukocyte infiltration into thrombi in rats

DVT rat models were administrated AS-IV or DMSO orally for 14 days after thrombosis induction. The representative images of thrombi are presented in Figure 2A. Thrombus weight and size were significantly increased after IVC stenosis, whereas AS-IV administration abolished this increase in tumor weight and size (Fig. 2B,C). Compared with sham-operated rats, DVT rat models showed augmented thrombosis in IVC, whereas administration of AS-IV attenuated the thrombosis (Fig. 2D). Leukocytes are associated with an increased risk of venous thrombosis (Kushnir et al., 2016). More leukocytes were recruited to thrombi after IVC stenosis. However, AS-IV administration markedly reduced the influxes of neutrophils, monocytes, and lymphocytes to thrombi (Fig. 2E-G). These results suggest that AS-IV treatment may inhibit thrombosis and suppress leukocyte infiltration into thrombi in rats.

AS-IV prevents the production of proinflammatory cytokines in rats

Leukocytes release proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 (Swystun and Liaw 2016). The serum levels of these proinflammatory cytokines were remarkably upregulated in experimental DVT rat models, whereas administration of AS-IV in DVT rats attenuated this promotion (Fig. 3A-C).

AS-IV inactivates PI3K/AKT signaling in rats

Mechanistically, IVC stenosis caused an increase in the phosphorylation of PI3K and AKT, whereas AS-IV treatment counteracted the pathway-activating effects of IVC stenosis, as western blotting revealed (Fig. 4A-C). Taken together, AS-IV deactivates PI3K/AKT signaling.

Discussion

DVT is a common life-threatening disorder with a significant mortality rate. In patients with proximal
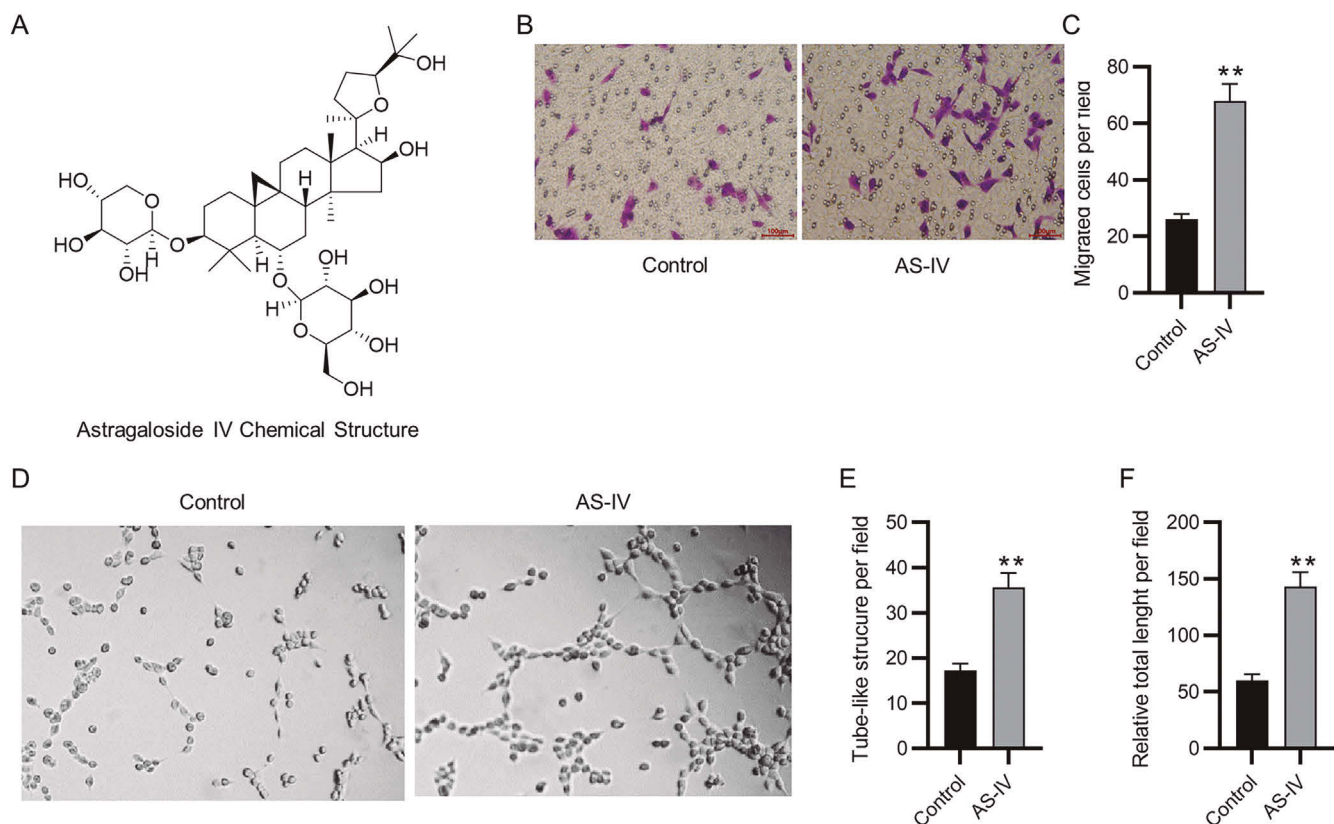

Fig. 1. AS-IV facilitates human EPC migration and angiogenesis. **A.** The chemical structure of AS-IV. **B, C.** Peripheral blood was collected from 10 patients with newly diagnosed DVT to isolate EPCs, and human EPCs at passage 3 were used. The migrative capability of human EPCs with or without AS-IV treatment was detected by Transwell assays. **D.** The *in vitro* tube formation assay was used to determine the angiogenesis ability of human EPCs. **E, F.** Quantification of tube formation and length. AS-IV, Astragaloside IV; EPC, endothelial progenitor cells. Data are expressed as mean ± standard deviation from three independent experiments. **p<0.01 compared with the Control group.
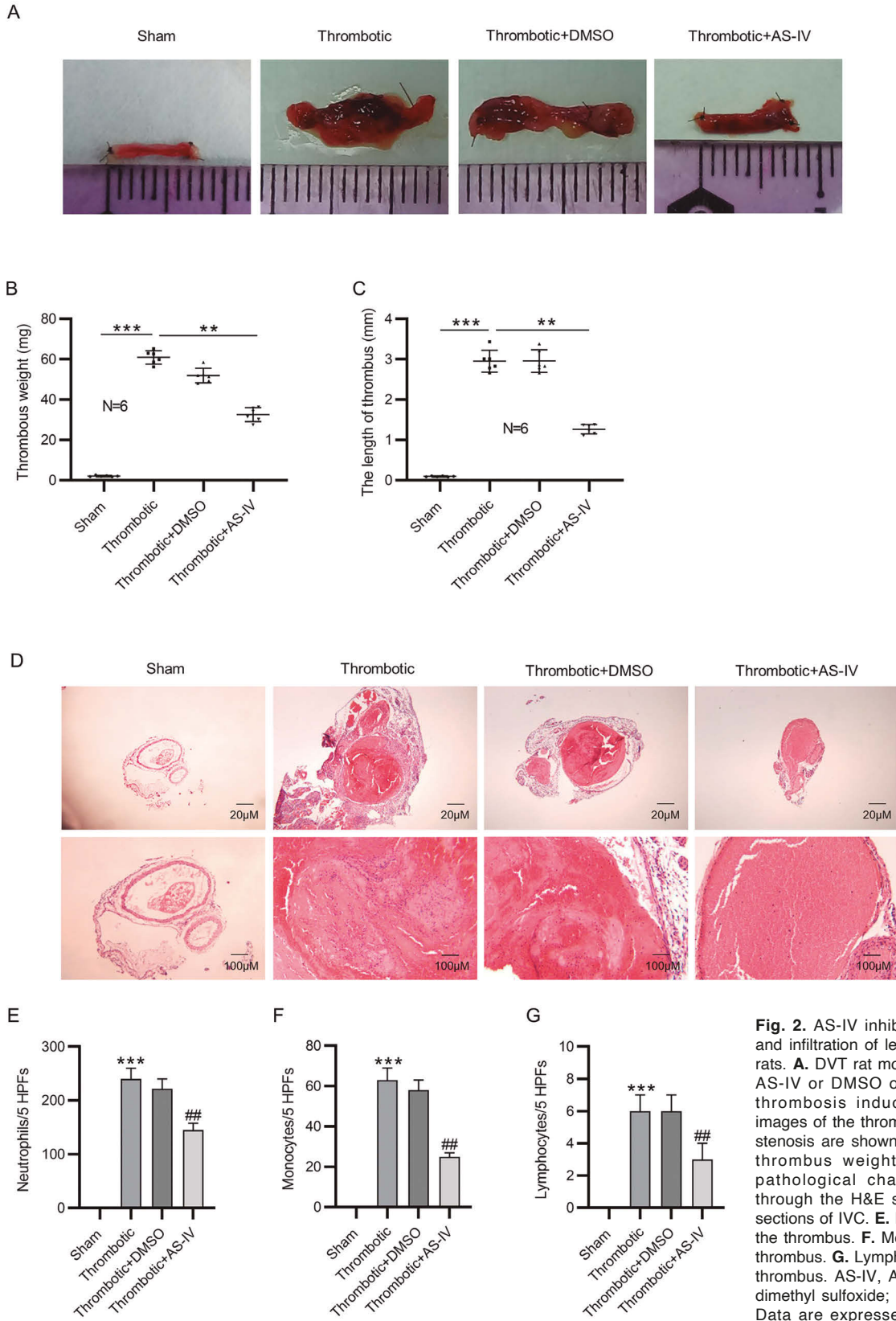
A

B

C

D

E

F

G

Fig. 2. AS-IV inhibits thrombus formation and infiltration of leukocytes into thrombi in rats. **A.** DVT rat models were administrated AS-IV or DMSO orally for 14 days after thrombosis induction. Representative images of the thrombus at day 14 after IVC stenosis are shown. **B, C.** Quantification of thrombus weight and length. **D.** The pathological changes were observed through the H&E staining of serial cross-sections of IVC. **E.** Neutrophil infiltration into the thrombus. **F.** Monocyte filtration into the thrombus. **G.** Lymphocyte infiltration into the thrombus. AS-IV, Astragaloside IV; DMSO, dimethyl sulfoxide; H&E, hematoxylin-eosin. Data are expressed as mean ± standard deviation from three independent experiments. N=6 for each group. ***$p<0.001$ compared with the Sham group, ##$p<0.01$ compared with the Thrombotic group.

DVTs, 40% are likely to develop pulmonary embolism. Death occurs in approximately 6% of DVT cases and 12% of pulmonary embolism cases within one month of diagnosis (Waheed et al., 2023). The existing therapeutic options have side effects. Thus, we explored novel agents for DVT treatment. AS-IV exerts protective effects against cardiovascular diseases, including heart dysfunction (Wang et al., 2021d), myocardial hypertrophy (Zhang et al., 2020), cardiomyocyte injury induced by ischemia (Huang et al., 2021a), hypoxia/reoxygenation (Yang et al., 2020a), and doxorubicin (Luo et al., 2021). However, the biological functions of AS-IV in DVT pathogenesis remain unknown. Herein, we found that AS-IV administration reduced thrombus weight and length and attenuated thrombosis in a rat model of DVT, then we investigated the mechanisms by which AS-IV mitigated thrombosis.

EPCs are critical in vascular intima repair, reendothelialization of denuded vessels, and angiogenesis, and their migration into thrombi leads to recanalization (Zilla et al., 2007). AS-IV reverses the inhibitory effects of high glucose on the migration of keratinocytes (Gao et al., 2022), promotes the migration of adipose-derived mesenchymal stem cells (Wang et al., 2022), enhances the migration and tube formation functions of high glucose-treated human umbilical vein endothelial cells (HUVECs) (Zou et al., 2022), and recovers the migrative ability of oxidized low-density lipoprotein-treated EPCs and HUVECs (Qian et al., 2019; Shao et al., 2021). Additionally, AS-IV improves the angiogenesis of EPCs around the wound (Huang et al., 2021b), promotes the angiogenesis of vascular endothelial cells in hypoxic conditions (Wang et al., 2021a), and enhances the angiogenesis of HUVECs after myocardial infarction (Cheng et al., 2019). This study showed that AS-IV administration recovered the migrative and tube formation abilities of EPCs, which was similar to previous findings.

The intricate relationship between inflammation and thrombosis is mediated by the endothelium, leukocytes,
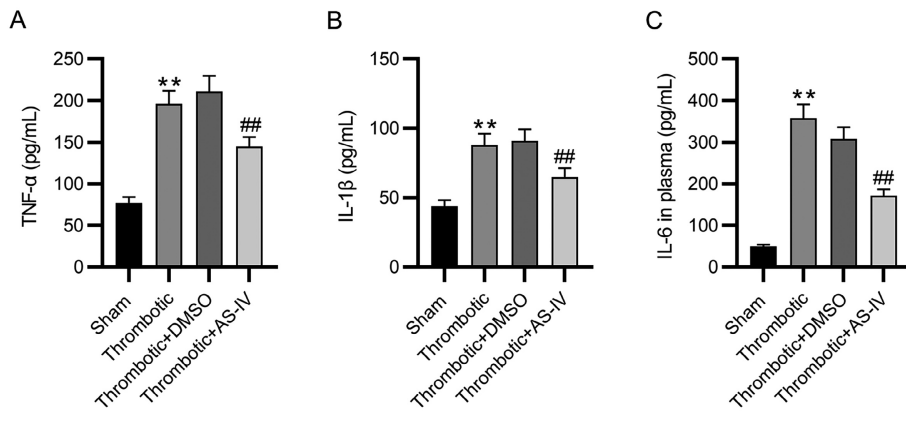

Fig. 3. AS-IV prevents the production of proinflammatory cytokines in rats. Serum level of TNF-α (A); IL-1β (B); and IL-6 (C). AS-IV, Astragaloside IV; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6. Data are expressed as mean ± standard deviation from three independent experiments. N=6 for each group. **$p<0.01$ compared with the Sham group, ##$p<0.01$ compared with the Thrombotic group.
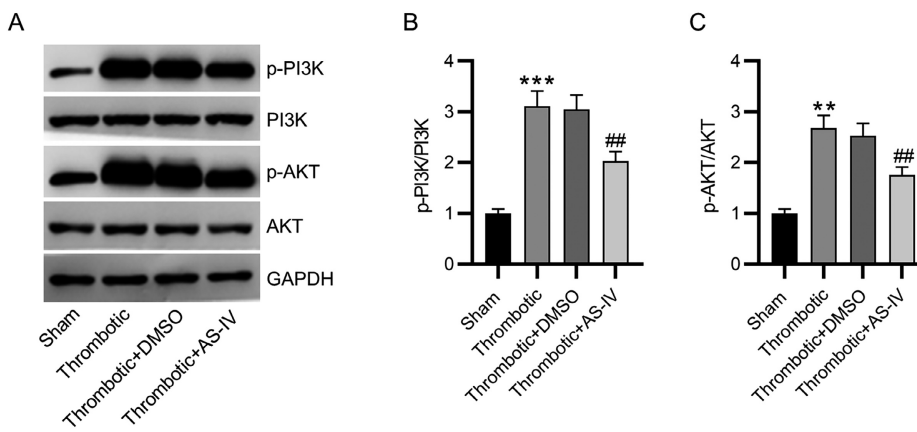

Fig. 4. AS-IV inactivates PI3K/AKT signaling in rats. A-C. The protein levels of p-PI3K, PI3K, p-AKT, and AKT in thrombi were measured by western blotting. AS-IV, Astragaloside IV; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B. Data are expressed as mean ± standard deviation from three independent experiments. N=6 for each group. **$p<0.01$, ***$p<0.001$ compared with the Sham group, ##$p<0.01$ compared with the Thrombotic group.

and platelets. Leukocytes are involved in venous thromboembolism, and leukocyte-derived particles are associated with increased thrombus formation (Galeano-Valle et al., 2021). AS-IV treatment reduces inflammatory cell infiltration and inflammatory cytokine production in experimental colitis (Zhong et al., 2022), myocardial ischemia/reperfusion injury (He et al., 2022), and asthma (Yang and Wang, 2019). The current study demonstrated that AS-IV administration decreased the infiltration of neutrophils, monocytes, and lymphocytes into the thrombus and prevented the release of TNF-α, IL-1β, and IL-6, indicating that AS-IV treatment relieved the IVC stenosis-induced inflammation in the thrombus and serum.

The PI3K/AKT pathway is suggested to regulate cell proliferation, cell apoptosis, and inflammatory response (Zhang et al., 2019). Inhibition of PI3K/AKT signaling is associated with attenuation of platelet aggregation and thrombus formation, while activation of PI3K/AKT induces endothelial damage, apoptosis, and inflammation (Chang et al., 2016; Su et al., 2016). AS-IV is found to inactivate the PI3K/AKT pathway to improve hematoma absorption after intracerebral hemorrhage and prevent PM2.5-induced lung injury (Pei et al., 2021; Zheng et al., 2022). Herein, AS-IV administration reduced the phosphorylation of PI3K and AKT, suggesting that AS-IV inactivated PI3K/AKT signaling.

In the present study, there are some limitations. First, although it seems that AS-IV could have some benefits in the treatment of DVT, these investigations have not yet been performed in humans, and the side effects of AS-IV are, as yet, unknown in humans. Second, different doses of AS-IV have not been tested. Third, we have not studied the effect of AS-IV on rats after PI3K/AKT inhibitors were used. Moreover, the value of adjuvant AS-IV during other thrombectomy methods in treating DVT should be addressed in the future.

In conclusion, this study demonstrates that AS-IV mitigates thrombosis in an experimental DVT model by promoting EPC angiogenesis and preventing inflammation *in vivo* through inactivating PI3K/AKT signaling. This study provides experimental data for the prevention and treatment of DVT and provides new avenues for further research.

References

Albricker A.C.L., Freire C.M.V., Santos S.N.D., Alcantara M.L., Saleh M.H., Cantisano A.L., Teodoro J.A.R., Porto C.L.L., Amaral S.I.D., Veloso O.C.G., Petisco A.C.G.P., Barros F.S., Lins de Barros M.V., de Souza A.J., Sobreira M.L., Barbosa de Miranda R., de Moraes D., Verrastro C.G.Y., Mançano A.D., de Souza Leão Lima R., Muglia V.F., Matushita C.S., Lopes R.W., Coutinho A.M.N., Pianta D.B., Damas Dos Santos A.A.S.M., de Lima Naves B., Vieira M.L.C. and Rochitte C.E. (2022). Joint guideline on venous thromboembolism - 2022. Arq. Bras. Cardiol. 118, 797-857.

Angona A., Alvarez-Larrán A., Bellosillo B., Martínez-Avilés L., Garcia-Pallarols F., Longarón R., Ancochea À. and Besses C (2015). Essential thrombocythemia: baseline characteristics and risk factors for survival and thrombosis in a series of 214 patients. Med. Clin. (Barc) 144, 247-253 (in Spanish).

Chang M.C., Chen Y.J., Liou E.J., Tseng W.Y., Chan C.P., Lin H.J., Liao W.C., Chang Y.C., Jeng P.Y. and Jeng J.H. (2016). 7-Ketocholesterol induces ATM/ATR, Chk1/Chk2, PI3K/Akt signalings, cytotoxicity and IL-8 production in endothelial cells. Oncotarget 7, 74473-74483.

Chen R., Feng R., Jiang S., Chang G., Hu Z., Yao C., Jia B., Wang S. and Wang S. (2022). Stent patency rates and prognostic factors of endovascular intervention for iliofemoral vein occlusion in post-thrombotic syndrome. BMC Surg. 22, 269.

Cheng S., Zhang X., Feng Q., Chen J., Shen L., Yu P., Yang L., Chen D., Zhang H., Sun W. and Chen X. (2019). Astragaloside IV exerts angiogenesis and cardioprotection after myocardial infarction via regulating PTEN/PI3K/Akt signaling pathway. Life Sci. 227, 82-93.

Feng Y., Lei B., Zhang F., Niu L., Zhang H. and Zhang M. (2017). Anti-inflammatory effects of simvastatin during the resolution phase of experimentally formed venous thrombi. J. Investig. Med. 65, 999-1007.

Fuchs T.A., Brill A. and Wagner D.D. (2012). Neutrophil extracellular trap (NET) impact on deep vein thrombosis. Arterioscler. Thromb. Vasc. Biol. 32, 1777-1783

Galeano-Valle F., Ordieres-Ortega L., Oblitas C.M., Del-Toro-Cervera J., Alvarez-Sala-Walther L. and Demelo-Rodríguez P. (2021). Inflammatory biomarkers in the short-term prognosis of venous thromboembolism: A narrative review. Int. J. Mol. Sci. 22, 2627.

Gao Q., Pan L., Li Y. and Zhang X. (2022). Astragaloside IV attenuates high glucose-induced human keratinocytes injury via TGF-β/Smad signaling pathway. J. Tissue Viability 31, 678-686.

He K., Yan L., Lin S.Q., Liu Y.Y., Hu B.H., Chang X., Zhao X.R., He S.Y., Wei X.H., Fan J.Y., Pan C.-S. and Han J.-Y. (2022).

Implication of IGF1R signaling in the protective effect of Astragaloside IV on ischemia and reperfusion-induced cardiac microvascular endothelial hyperpermeability. Phytomedicine 100, 154045

Hou Y. and Li C. (2018). Stem/Progenitor cells and their therapeutic application in cardiovascular Disease. Front. Cell Dev. Biol. 6, 139.

Hsieh H.L., Liu S.H., Chen Y.L., Huang C.Y. and Wu S.J. (2022). Astragaloside IV suppresses inflammatory response via suppression of NF-κB, and MAPK signalling in human bronchial epithelial cells. Arch. Physiol. Biochem. 128, 757-766

Huang K.Y., Yu Y.W., Liu S., Zhou Y.Y., Wang J.S., Peng Y.P., Ji K.T. and Xue Y.J. (2021a). A single, acute astragaloside IV therapy protects cardiomyocyte through attenuating superoxide anion-mediated accumulation of autophagosomes in myocardial ischemia-reperfusion injury. Front. Pharmacol. 12, 642925

Huang N.C., Dai L.G., Kang L.Y., Huang N.C., Fu K.Y., Hsieh P.S. and Dai N.T. (2021b). Beneficial effects of astragaloside IV-treated and 3-dimensional-cultured endothelial progenitor cells on angiogenesis and wound healing. Ann. Plast. Surg. 86, S3-s12.

Kim M., Kwon H., Hong S.K., Han Y., Park H., Choi J.Y., Kwon T.W. and Cho Y.P. (2015). Surgical treatment of central venous catheter related septic deep venous thrombosis. Eur. J. Vasc. Endovasc. Surg. 49, 670-675.

Kong L., Du X., Hu N., Li W., Wang W., Wei S., Zhuang H., Li X. and Li C. (2016a). Downregulation of let-7e-5p contributes to endothelial progenitor cell dysfunction in deep vein thrombosis via targeting FASLG. Thromb. Res. 138, 30-36.

Kong L., Hu N., Du X., Wang W., Chen H., Li W., Wei S., Zhuang H., Li X. and Li C. (2016b). Upregulation of miR-483-3p contributes to endothelial progenitor cells dysfunction in deep vein thrombosis patients via SRF. J. Transl. Med. 14, 23.

Kushnir M., Cohen H.W. and Billett H.H. (2016). Persistent neutrophilia is a marker for an increased risk of venous thrombosis. J. Thromb. Thrombolysis 42, 545-551.

Li W.D. and Li X.Q. (2016). Endothelial progenitor cells accelerate the resolution of deep vein thrombosis. Vascul. Pharmacol. 83, 10-16.

Liu D., Zhu Y., Chen W., Li J., Zhao K., Zhang J., Meng H. and Zhang Y. (2020a). Relationship between the inflammation/immune indexes and deep venous thrombosis (DVT) incidence rate following tibial plateau fractures. J. Orthop. Surg. Res. 15, 241.

Liu X., Shang S., Chu W., Ma L., Jiang C., Ding Y., Wang J., Zhang S. and Shao B. (2020b). Astragaloside IV ameliorates radiation-induced senescence via antioxidative mechanism. J. Pharm. Pharmacol. 72, 1110-1118.

Lo Gullo A., Aragona C.O., Scuruchi M., Versace A.G., Saitta A., Imbalzano E., Loddo S., Campo G.M. and Mandraffino G. (2018). Endothelial progenitor cells and rheumatic disease modifying therapy. Vascul. Pharmacol. 108, 8-14.

Luo L.F., Qin L.Y., Wang J.X., Guan P., Wang N. and Ji E.S. (2021). Astragaloside IV attenuates the myocardial injury caused by adriamycin by inhibiting autophagy. Front. Pharmacol. 12, 669782.

Miller-Kasprzak E. and Jagodziński P.P. (2007). Endothelial progenitor cells as a new agent contributing to vascular repair. Arch. Immunol. Ther. Exp. (Warsz) 55, 247-259.

Naringrekar H., Sun J., Ko C. and Rodgers S.K. (2019). It's not all deep vein thrombosis: Sonography of the painful lower extremity with multimodality correlation. J. Ultrasound Med. 38, 1075-1089.

Parker K. and Thachil J. (2018). The use of direct oral anticoagulants in chronic kidney disease. Br. J. Haematol. 183, 170-184.

Pei C., Wang F., Huang D., Shi S., Wang X., Wang Y., Li S., Wu Y. and Wang Z. (2021). Astragaloside IV protects from PM2.5-induced lung injury by regulating autophagy via inhibition of PI3K/Akt/mTOR signaling *in vivo* and *in vitro*. J. Inflamm. Res. 14, 4707-4721.

Qian W., Cai X., Qian Q., Zhuang Q., Yang W., Zhang X. and Zhao L. (2019). Astragaloside IV protects endothelial progenitor cells from the damage of ox-LDL via the LOX-1/NLRP3 inflammasome pathway. Drug Des. Devel. Ther. 13, 2579-2589.

Qin L.Y., Guan P., Wang J.X., Chen Y., Zhao Y.S., Yang S.C., Guo Y.J., Wang N. and Ji E.S. (2022). Therapeutic potential of Astragaloside IV against adriamycin-induced renal damage in rats via ferroptosis. Front. Pharmacol. 13, 812594.

Ren S., Zhang H., Mu Y., Sun M. and Liu P. (2013). Pharmacological effects of Astragaloside IV: a literature review. J. Tradit. Chin. Med. 33, 413-416.

Schrottmaier W.C., Mussbacher M., Salzmann M. and Assinger A. (2020). Platelet-leukocyte interplay during vascular disease. Atherosclerosis 307, 109-120.

Seifi A., Dengler B., Martinez P. and Godoy D.A. (2018). Pulmonary embolism in severe traumatic brain injury. J. Clin. Neurosci. 57, 46-50.

Shao X., Liu Z., Liu S., Lin N. and Deng Y. (2021). Astragaloside IV alleviates atherosclerosis through targeting circ_0000231/miR-135a-5p/CLIC4 axis in AS cell model *in vitro*. Mol. Cell. Biochem. 476, 1783-1795.

Su X.L,. Su W., Wang Y., Wang Y.H., Ming X. and Kong Y. (2016). The pyrrolidinoindoline alkaloid Psm2 inhibits platelet aggregation and thrombus formation by affecting PI3K/Akt signaling. Acta Pharmacol. Sin. 37, 1208-1217.

Swystun L.L. and Liaw P.C. (2016). The role of leukocytes in thrombosis. Blood 128, 753-762.

Waheed S.M., Kudaravalli P. and Hotwagner D.T. (2023). Deep vein thrombosis. In: StatPearls. StatPearls Publishing.

Wang B., Zhang C., Chu D., Ma X., Yu T., Liu X. and Hu C. (2021a). Astragaloside IV improves angiogenesis under hypoxic conditions by enhancing hypoxia-inducible factor-1α SUMOylation. Mol. Med. Rep. 23, 244.

Wang B.S., Ma X.F., Zhang C.Y., Li Y.X., Liu X.Z. and Hu C.Q. (2021b). Astragaloside IV improves angiogenesis and promotes wound healing in diabetic rats *via* the activation of the SUMOylation pathway. Biomed. Environ. Sci. 34, 124-129.

Wang F., Qian H., Kong L., Wang W., Wang X., Xu Z., Chai Y., Xu J. and Kang Q. (2021c). Accelerated bone regeneration by Astragaloside IV through stimulating the coupling of osteogenesis and angiogenesis. Int. J. Biol. Sci. 17, 1821-1836.

Wang Q., Yang X., Song Y., Sun X., Li W., Zhang L., Hu X., Wang H., Zhao N., Zhuang R. Xie X., Tang F. and Wang H. (2021d). Astragaloside IV-targeting miRNA-1 attenuates lipopolysaccharide-induced cardiac dysfunction in rats through inhibition of apoptosis and autophagy. Life Sci. 275, 119414.

Wang W., Shen Z., Tang Y., Chen B., Chen J., Hou J., Li J., Zhang M., Liu S., Mei Y., Zhang L. and Lu S. (2022). Astragaloside IV promotes the angiogenic capacity of adipose-derived mesenchymal stem cells in a hindlimb ischemia model by FAK phosphorylation via CXCR2. Phytomedicine 96, 153908.

Wu Y.J., Li Z.H., Li J.Y., Zhou Y., Wang R.Y., Chen X.Y., Qing L.S. and Luo P. (2022). Elucidation of the binding mechanism of astragaloside IV derivative with human serum albumin and its cardiotoxicity in zebrafish embryos. Front. Pharmacol. 13, 987882.

Yang X. and Wang F. (2019). The effect of astragaloside IV on JAK2-STAT6 signalling pathway in mouse model of ovalbumin-induced asthma. J. Anim. Physiol. Anim. Nutr. (Berl) 103, 1578-1584.

Yang J.J., Zhang X.H., Ma X.H., Duan W.J., Xu N.G., Chen Y.J. and Liang L. (2020a). Astragaloside IV enhances GATA-4 mediated myocardial protection effect in hypoxia/reoxygenation injured H9c2 cells. Nutr. Metab. Cardiovasc Dis. 30, 829-842.

Yang X., Song Y., Sun Y., Wang M. and Xiang Y. (2020b). Down-regulation of miR-361-5p promotes the viability, migration and tube formation of endothelial progenitor cells via targeting FGF1. Biosci. Rep. 40, BSR20200557.

Zhang Y., Du X., Li W., Sang H., Qian A., Sun L., Li X. and Li C. (2018). Resveratrol improves endothelial progenitor cell function through miR-138 by targeting focal adhesion kinase (FAK) and promotes thrombus resolution *in vivo*. Med. Sci. Monit. 24, 951-960.

Zhang H.B., Tu X.K., Chen Q. and Shi S.S. (2019). Propofol reduces inflammatory brain injury after subarachnoid hemorrhage: Involvement of PI3K/Akt pathway. J. Stroke Cerebrovasc. Dis. 28, 104375.

Zhang T., Wang H., Lu M., Zhao K., Yin J., Liu Y. and Sun Y. (2020). Astragaloside IV prevents myocardial hypertrophy induced by mechanical stress by activating autophagy and reducing inflammation. Am. J. Transl. Res. 12, 5332-5342.

Zheng Y., Li R., Zhou Y., Zhang S. and Fan X. (2022). Investigation on the potential targets of Astragaloside IV against intracerebral hemorrhage based on network pharmacology and experimental validation. Bioorg. Chem. 127, 105975.

Zhong Y., Liu W., Xiong Y., Li Y., Wan Q., Zhou W., Zhao H., Xiao Q. and Liu D. (2022). Astragaloside IV alleviates ulcerative colitis by regulating the balance of Th17/Treg cells. Phytomedicine 104, 154287.

Zilla P., Bezuidenhout D. and Human P. (2007). Prosthetic vascular grafts: wrong models, wrong questions and no healing. Biomaterials 28, 5009-5027.

Zou X., Xiao H., Bai X., Zou Y., Hu W., Lin X., Zhu C., Liang Y. and Xiong W. (2022). Astragaloside IV drug-loaded exosomes (AS-IV EXOs) derived from endothelial progenitor cells improve the viability and tube formation in high-glucose impaired human endothelial cells by promoting miR-214 expression. Endokrynol. Pol. 73, 336-345.