Garcinoic Acid Improves Cardiac Function and Enhances Angiogenesis Following Myocardial Infarction

Hongyao Hu (✉ huhongyao@whu.edu.cn)

Department of Interventional Radiology, Department of Radiology, Renmin Hospital of Wuhan University

Wei Li

Department of Cardiology, Renmin Hospital of Wuhan University

Yanzhao Wei

Department of Cardiology, Renmin Hospital of Wuhan University

Hui Zhao

Department of Interventional Radiology, Department of Radiology, Renmin Hospital of Wuhan University

Zhenzhong Wu

Department of Interventional Radiology, Department of Radiology, Renmin Hospital of Wuhan University

Min Rao

Department of Interventional Radiology, Department of Radiology, Renmin Hospital of Wuhan University

Yanhong Tang

Department of Cardiology, Renmin Hospital of Wuhan University

---

Research Article

Abstract

Cardiac ischemia impairs angiogenesis in response to hypoxia, resulting in ventricular remodeling. Garcinoic acid (GA), the extraction from the plant garcinia kola, is validated to attenuate inflammatory response. However, the role of GA in heart failure (HF) and neovascularization after myocardial infarction (MI) is incompletely understood. The present study is striving to explore the role of GA and the potential mechanism of which in cardiac function after MI. SD rats were randomized into sham group, MI+vehicle group, and MI+GA group in vivo. Human umbilical endothelial cells (HUVECs) were cultured in vehicle or GA, and then additionally exposed to 2% hypoxia environment in vitro. MI rats displayed a dramatically reduced myocardial injury, cardiac function and vessel density in the peri-infarcted areas. GA delivery markedly improved cardiac performance and promoted angiogenesis. In addition, GA significantly enhanced tube formation in HUVECs under hypoxia condition. Furthermore, the expressions of pro-angiogenic factors HIF-1α, VEGF-A and bFGF, and pro-angiogenic proteins phospho-VEGFR2Tyr1175 and VEGFR2, as well as phosphorylation levels of Akt and eNOS were increased by GA treatment. In conclusion, GA preserved cardiac function after MI probably via promoting neovascularization. And the potential mechanism may be partially through upregulating the expressions of HIF-1α, VEGF-A, bFGF, phospho-VEGFR2Tyr1175 and VEGFR2 and activating the phosphorylations of Akt and eNOS.

1. Introduction

Acute myocardial infarction (MI) is one of the chief causes of global morbidity and mortality (Cao *et al.*, 2014). Occlusion of coronary arteries, which leads to insufficient oxygen and nutrient supply, is the major pathology of MI and it is by which the ischemia progress into necrosis. Restoration of myocardial blood supply in necrotic or peri-infarct areas as soon as possible is beneficial to myocardial repair after MI (Shah & Mann, 2011). Early reperfusion of the acute occlusive coronary artery greatly improves the prognosis of patients with MI, thereby reducing myocardial necrosis (Yellon & Hausenloy, 2007). This strategy is regarded as the most effective method for the treatment of acute MI in clinical studies. However, it needs more justification whether such treatments towards microvascular rarefaction and cardiac dysfunction in the ischemic heart can contribute to an effective myocardial ischemia-reperfusion. Proangiogenic therapy has theoretically appeared a promising strategy for anti-ventricular remodeling after MI, but current clinical trials reported little positive news (Tongers *et al.*, 2011). Notwithstanding we face such a dilemma in vascular regeneration therapy, the challenge must be overcome before therapeutic angiogenesis becomes a pragmatic therapeutic methodology.

In the early stage of inflammation, macrophages and neutrophils clear necrotic cardiomyocytes and promote the proliferation of endothelial cells (ECs) and fibroblasts to form vascularized granulation tissue, but then it can cause apoptosis of these cells to form collagen-rich scars, resulting in microvascular rarefaction (Frangogiannis, 2006). Inhibition of inflammatory responses can significantly enhance healing after MI by increasing neovascularization in peri-infarcteded areas (Tang *et al.*, 2017; Ferraro *et al.*, 2019). Extractions of Garcinia kola seeds have the properties of anti-oxidation and anti-inflammation (Adedara *et al.*, 2015; Kenne Michel *et al.*, 2015). Garcinia kola can also attenuate

doxorubicin-induced cardiotoxicity and protect against ischaemia/reperfusion injury (Oyagbemi *et al.*, 2017; Oyagbemi *et al.*, 2018). In addition, recent researches have demonstrated that purified garcinoic acid (GA) from Garcinia kola nut seeds, as a major bioactive component in the seed, has an anti-inflammatory effect and can inhibit the occurrence of atherosclerosis (Wallert *et al.*, 2019). However, up to now, it is unknown whether administration of GA has an cardioprotective effect on cardiac dysfunction and neovascularization after myocardial ischemia.

In the present study, we investigated the role of GA in myocardial angiogenesis of peri-infarcteded areas and cardiac function„as well as examined the potential mechanisms through both vivo and vitro methods.

2. Materials And Methods

2.1.Experimental animals and treatments

All adult male Sprague-Dawley (SD) rats (weighing 180-200 g) were acquired from the Animal Center of the Renmin Hospital of Wuhan University. All animal care and experimental procedures were approved by the Guidelines for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (revised 2011) and in compliance with the Animal Care and Use Committee of Renmin Hospital of Wuhan University. All rats were maintained under specific pathogen-free (humidity 50 ± 5%; temperature 20-22 °C) with a 12-hour light/dark cycle.

Rats were subjected to either a ligation of left anterior descending branch to make MI model or a sham operation according with previous method (Yuan *et al.*, 2016a). The rats were randomly divided into three groups: a sham operation group (sham), a MI group (MI+vehicle) and a garcinoic acid treatment (GA)+MI group (GA+MI). We administered GA (1 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) or vehicle (PBS+DMSO) by intraperitoneal (i.p.) injection weekly for 2 weeks (Wallert *et al.*, 2019). All the specimens were collected from the peri-infarcted areas (< 2 mm outside the infarct).

2.2.Echocardiography

Echocardiography was conducted 2 weeks after MI. According to previous study, after anesthetized with 1.5-2% isoflurane, transthoracic echocardiography was performed by a Mylab30CV ultrasound (Esaote SpA, Genoa, Italy) using a 15-MHz imaging transducer (Yuan *et al.*, 2016b). Then, the parameters of cardiac function including left ventricle (LV) end-diastolic diameter (LVEDD), ejection fraction (EF), fractional shortening (FS) were collected.

2.3. Cell culture and treatments

Human umbilical endothelial cells (HUVECs; Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were used for all in vitro experiments. HUVECs were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS,GIBCO,USA) / 1% penicillin-streptomycin (100 U/mL) in a cell incubator with humidified 95% O_2 and 5% CO_2 atmosphere at 37°C for normoxia condition. Hypoxia condition was performed with hypoxic gas mixture (2% O_2, 5% CO_2 and 93% N_2) from cell incubator. Before hypoxia, HUVECs were pretreated in different concentrations of GA (1μM, 2.5μM and 5μM) or vehicle for 24 hours.

2.4.Cell viability

The cell viability was evaluated by the Cell Counting kit (CCK-8) assay (Dojindo, GB707, Japan). The cells were treated with various concentrations of GA and vascular endothelial growth factor (VEGF) and/or hypoxia and incubated with 10 μL CCK8 solution on a 96-well microplate reader for 2 hours,.The absorbance was detected at 450 nm wavelength by an ELISA reader.

2.5.Tube formation assays

After allowing the BD Matrigel^{TM} Basement Membrane Matrix to settle for 30 min in a 37°C, 5% CO2 incubator, HUVECs from different groups were seeded on the layer of polymerized gel on a 96-well plate, hypoxia+VEGF was used as a positive control. 12 hours after incubation, the formation of a capillary-like structure was recorded using microscope and images taken by light microscopy (ECLIPSE 80i; Nikon, Japan). Tube formation was quantified by using image-analysis software (Image J, version 1.40g, NIH).

2.6.Enzyme linked-immunosorbent assay (ELISA)

Plasma creatinine kinase-MB (CK-MB; Jiancheng Bioengineering Institute, Nanjing, China) and cardiac troponin I (cTnI; Life Diagnostic, Pennsylvania, USA) from jugular vein blood at various time points (0, 7 and 14day) were determined with ELISA detection kits as per manufacturer's instructions. After treatment for 2 weeks, the serum was collected to detect the concentration of BNP with a BNP ELISA detection kit (BD Biosciences, San Jose, CA) according to the manufacturer's instructions.

2.7.Masson's Trichrome

Maximum section of heart was fixed in 4% formaldehyde for 24h, after which it was embedded in paraffin, and cut into 5 μm thick slices. The slices were stained with Masson trichrome staining for evaluation of infarcted size in the heart. The fibrosis areas were observed using a light microscope (Olympus D72, Japan) .The infarcted size was calculated as the percentage of the positively stained area of the total myocardial tissue, and then analyzed by using Image J software.

2.8. Immunofluorescence analysis

The dissected hearts in the peri-infarcteded areas were fixed in 4% phosphate-buffered formalin, and then the samples were embedded in paraffin to cut into 5 μm sections. To evaluate vessel density and proliferation, alpha smooth muscle actin (α-SMA)/CD31 and ki67/CD31 co-immunostaining was performed. After blocking with 10% goat serum, the sections were incubated in α-SMA, ki67 and CD31 primary antibody (Abcam, Cambridge, UK) overnight and subsequently incubated with Alexa-Fluor-coupled secondary antibodies, then counterstained with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen, Carlsbad, CA, USA). The images were obtained by using a fluorescence microscope (OLYMPUS, Tokyo, Japan) and then analyzed by using Image J software.

2.9. Western blot

Total proteins from rat hearts or cultured HUVECs were extracted by use of RIPA lysis buffer supplemented with protease/phosphatase inhibitor cocktail (CST, Danvers, MA, USA). Equal amount (50 μg) of protein lysate was subjected to 10% SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, MA, USA). After blocking with 5% non-fat milk, the membrane was incubated overnight with the following primary antibody: HIF-1α (sc-13515, Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA), VEGF-A (ab46154, Abcam), basic fibroblast growth factor (bFGF; NB600-1536, Novus Biologicals, CO, USA▯▯ phosphorylated VEGF receptor 2 (p-VEGFR2) (SAB4504567, Sigma), VEGFR2 (SAB4501642, Sigma), Ser^{473} -p-Akt (#4060, CST), p-Akt (#9272, CST), Ser1177- p-eNOS (ab138458, Abcam), eNOS (ab199956, Abcam), GAPDH (ab37168, Abcam). Next, the membranes were washed and incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody. Proteins were treated with an ECL detection kit and then visualized using chemiluminescence system. The specific protein expression levels were normalized to GAPDH.

2.10. Statistical analysis

The data analyses were performed with the GraphPad Prism 6.0. All data were expressed as mean ± SEM. Comparisons for multiple-group were analyzed using ANOVA with the Tukey's test. A value of $P < 0.05$ was deemed statistically significant.

3. Results

3.1. GA treatment reduces myocardial injury and promotes recovery of cardiac function after MI

To determine the effects of GA on infarcted size, Masson-trichrome staining was performed, the result showed that the infarct size was not obviously reduced with GA treatment in post-MI rats (Fig. 1A, B).

Furthermore, the levels of CK-MB and cTnI was considerably higher in the MI group at the 7 day post-MI, whereas GA significantly decreased this effect, but no significant difference was observed between the MI and GA+MI groups at the 14 day post-MI (Fig. 1C, D), suggesting a reduction in cardiac injury post-MI to some extent. To clarify whether GA therapy could improve cardiac function in MI rats, echocardiographic examination was carried out at day 14 after intervention. Compared with the sham groups, MI groups markedly induced cardiac dysfunction, as indicated by the distensible LVEDD and decreased EF and FS, which were partially restored to normal cardiac contractility and LV geometry in GA+MI supplement groups (Fig. 1E-H). Furthermore, cardiac dysfunction was also assessed by the serum levels of BNP by Elisa analysis, and we observed that GA could attenuate the MI-induced upregulation of BNP levels (Fig. 1I). Therefore, we concluded that GA treatment alleviated MI-induced myocardial necrosis and cardiac dysfunction in rats.

3.2.GA therapy boosts post-MI angiogenesis

Cardiac vessel density in the border zone was quantified with percentage of positive areas 14 days after operation through immunohistochemistry double-staining with α-SMA (a smooth muscle marker) and CD31 (an endothelial cell marker), Ki67 (a cell proliferation marker) and CD31. As expected, evidenced by the number of positive cells of CD31 (CD31$^+$), as well as α-SMA$^+$/ CD31$^+$ cells, MI rats showed a significant vessel density rarefaction in peri-infarcteded areas compared with sham groups whereas administration of GA increased the density of the mature blood vessels post-MI (Fig. 2A, C, D). Cell nuclear antigens Ki67and CD31 were co-immuostained to determine endothelial proliferation. The number of Ki67$^+$ cells and Ki67$^+$/ CD31$^+$ was significantly higher in the group receiving GA than that in the group receiving vehicle (Fig. 2B, E, F). These data indicated that neovascularization in the border zone of hearts at day 14 after MI was enhanced by GA treatment.

3.3.GA upregulates the expression of pro-angiogenic proteins and p-Akt, p-eNOS in post-MI hearts

To elucidate the underlying mechanisms, we examined the effects of GA on the expression levels of several pro-angiogenic proteins including HIF-1α, VEGF-A and bFGF, and related signaling molecules p-Akt and p-eNOS. I*n vivo* study, compared with the sham groups, MI groups had no effect on the production of HIF-1α, VEGF-A and bFGF in peri-infarcted areas of hearts. Treatment with GA induced a significant increase of these proteins compared with the vehicle-treated MI groups (Figs. 3A-D). Despite a slight increase of p-VEGFR2 (Tyr1175), it had no significant downregulation in the peri-infarcted myocardium of MI rats compared to sham rats. Treatment with GA markedly enhanced VEGFR2 phosphorylation. Furthermore, GA could increase the expression of VEGFR2 in infarcted hearts (Figs. 3E-G). The pro-angiogenic protein p-Akt was significantly upregulated in MI rats compared with the sham groups, while p-eNOS had no difference in the two groups. However, GA treatment stimulated the expressions of both p-Akt and p-eNOS (Figs 3H-J). Thus, vivo study showed that GA enhanced the phosphorylation of Akt and

eNOS through upregulation of HIF-1α, VEGF-A and bFGF, the effect of which possibly promoted post-MI neovascularization.

3.4.GA enhances the tube formation ability of HUVECs subjected to hypoxia

Initially, to confirm the possible cytotoxicity of GA towards HUVECs, we assessed cell viability using a CCK8 assay in exist of GA and/or hypoxia. We found that pretreatment with different concentration of GA for 30 h has no significant effect on HUVECs viability under normoxia condition. As expected, VEGF which served as a positive control boosted cell proliferation, (Fig. 4A). As shown in Fig. 4B, hypoxia for 6 h can induce the decline of cell activities. An obvious increase in cell proliferation was observed in GA-treated (2.5μM and above) cells in the presence of hypoxia. *In vitro*, HUVECs tubule formation ability was significantly depressed when HUVECs were subjected to hypoxia in Matrigel culture, whereas GA (2.5μM and above) could notably promote the formation of a tubular capillary-like network (Fig. 4C, D). The results illustrated that GA made for the formation of vascular tube-like structure under ischemia and hypoxia condition.

3.5.GA activates the expressions of angiogenesis proteins *in vitro* possibly via enhancing the Akt/eNOS signaling pathway

We further illuminated the mechanisms underlying pro-angiogenic phenomenon *in vitro* assay. Consistent with above methods, we pretreated HUVECs with different concentrations of GA for 24h, and then cultured them in hypoxia or normoxia for 6 h. The data showed that hypoxia induced higher HIF-1α and VEGF-A expressions in hypoxia HUVECs than in vehicle, while the expression of bFGF presented no significant difference between hypoxia and vehicle groups. As expected, the production of HIF-1α, VEGF-A and bFGF was further dramatically augmented by different concentrations of GA treatment，which had different efficacy, though(Fig. 5A-D). Exposure to GA rapidly resulted in a striking increase in phosphorylation of VEGFR2 within only 10 min. With the prolongation of time, the degree of p-VEGFR2 was attenuated (Fig. 5E, F). Pre-treatment with the GA (2.5 μM) pronouncedly increased the expression of VEGFR2 in hypoxia conditions (Figs. 5G, H). In addition, phosphorylation of Akt was notably activated in the hypoxia groups compared with the vehicle groups. Besides, p-eNOS did not significantly appear in hypoxia-treated HUVECs. The supply of GA further enhanced the degree of Akt and eNOS phosphorylation (Fig. 5I-K). Consistent with the vivo alterations, we found that the upregulation of HIF-1α, VEGF-A and bFGF affected by GA treatment was associated with activating p-Akt and p-eNOS in vitro study.

4. Discussion

In the present study, we observed that GA alleviated myocardial injury and cardiac dysfunction and promoted vascular regeneration in the infarcted border zone following MI. Supplementation of GA raised the endothelial cells viability and boosted tube formation in hypoxia-treated HUVECs. Furthermore, we also provided evidences that HIF-1α, VEGF-A, bFGF, and p-Akt/p-eNOS signaling pathways played an essential role in the angiogenesis, Therefore, our current findings approved that GA might be an effective therapeutic agent against cardiac injury.

Inflammatory response, compensatory hypertrophy of muscle and imbalance of vascular regression in the myocardium play an important role in cardiac injury and repair (Shiojima *et al.*, 2005; Liehn *et al.*, 2011). Promoting inflammatory cells release anti-inflammatory cytokines and angiogenic factors, which are critical to vanish the inflammation and promote angiogenesis (Nahrendorf *et al.*, 2007; Vannella & Wynn, 2017). It is well established that neovascularization in the peri-infarcted areas after MI is conducive to preserve cardiac function, thus reducing adverse left ventricular remodeling (Frangogiannis, 2012). Actually, except for delivering oxygen and nutrients, the crosstalk of endothelial cell/cardiomyocyte also regulates the cardiac contractility, giving rise to the protection of cardiomyocytes after ischemia in an NO-independent manner (Winegrad *et al.*, 1998; Leucker *et al.*, 2011). As expected, GA, an extraction from Garcinia kola nut, enhanced vascular density and angiogenesis in peri-infarcted areas of hearts post-MI. Moreover, considering that ECs proliferation is indispensable in the early stage of sprouting angiogenesis, we underwent cell viability assay *in vitro.*. And the result is in line with the proangiogenic effect of GA treatment *in vivo*. The protective effect of GA on cardiac function after MI is probably due to its already well-defined anti-inflammatory effect and its pro-angiogenic effect found in the present study.

Regulating specific transcriptional responses of mammalian cells to hypoxia is overwhelmingly mediated by HIF-1 which is composed of an HIF-1α and an HIF-1β subunit (Wang *et al.*, 1995). HIF-1α, as an upstream initiator of hypoxia and ischemia-induced angiogenesis, mediates cellular adaptation to hypoxia and regulates angiogenesis and therein has been demonstrated to be a pivotal regulator for gene expression, including those encoding angiogenesis cytokines such as VEGF and bFGF. (Pugh & Ratcliffe, 2003; Calvani *et al.*, 2006). Meanwhile some studies have demonstrated that VEGF treatment promotes neovascularization, increases vessel density, and ameliorates post-MI myocardial repair (Giacca & Zacchigna, 2012; Cheng *et al.*, 2013). The presence of HIF-1α-bFGF pathway enhances survival and sprouting of ECs under hypoxic conditions. Spatiotemporal delivery of bFGF promotes cardiac tissue vascularization and improves cardiac function following MI (Calvani *et al.*, 2006; Fan *et al.*, 2019). Intriguingly, our data confirmed that GA can promotes the expression of HIF-1α, VEGF and bFGF *in vivo* and *in vitro* experiments. Previous studies have demonstrated that VEGFR-2 played a vital role in angiogenesis and activation of VEGFR-2 promoted ECs growth, migration and tube formation. VEGF-A, as a crucial pro- angiogenic factor through VEGFR2 signal pathway can awake a variety of biological activities, including endothelial cells maturation, angiogenesis and arteriogenesis in response to hypoxia or healing.(Ferrara *et al.*, 2003; Jin *et al.*, 2018). Upregulation of these pro-angiogenic proteins was probably the main reason why GA can promote endothelial cell morphogenesis in *vitro* and angiogenesis *in vivo*.

Akt kinases regulate cellular survival, proliferation, metabolism and have been affirmed to play a vital role in revascularization (Chen *et al.*, 2005). Consistent with previous reports, our data showed that the degree of phosphorylation of Akt increased in 2 weeks after MI (Rengo *et al.*, 2012). While further upregulated p-Akt was one of the reasons for the increase of phosphorylation of eNOS. Endothelial NO synthase activation can induce enhancement of cardiac angiogenesis. Indeed, activated VEGF/Akt/eNOS pathway, which occurs within 2 weeks from therapy origination, is vital to the proangiogenic effects (Rengo *et al.*, 2012; Rengo *et al.*, 2013). Previous study suggested that garcinia kola seed extract can activate Akt phosphorylation in an ischemic/reperfusion injury rat heart model (Oyagbemi *et al.*, 2018). Besides, rusults in approximate agreement with previous reports, we also observed that GA increased the expression of p-Akt by verifying it from *in vivo* and in *vitro* experiments, as a consequence of which the downstream effectors eNOS is upregulated. Combined with recent other studies, we inferred that GA could be an effective pro-angiogenic drug in myocardial injury ultimately through Akt/eNOS activation.

In summary, the present study showed that GA treatment attenuated cardiac dysfunction and contributed to neovascularization in MI model through participating in the production of HIF-1α, VEGF and bFGF and the activation of p-Akt/eNOS. Thus, GA might be regarded as a candidate in the prevention and treatment of ischemic heart diseases.

Declarations

Ethics approval and consent to participate

All the adult male Sprague-Dawley rats in vivo and ex vivo experiments(weighing 180-220g) were purchased from Animal Center of Wuhan University.All the operations are in conformity with the Guide for the Care and Use of Laboratory Animal proposed by United States National Institude of Health(the Eighth Edition, National Research Council 2011)..All the participated animals are anesthetized before operation and properly dealt with after sacrifice.

Consent for publication

I understand and agree that BMC cardiovascular disorder is an open access journal and other publication policies.

Availability of data and materials

The data are not curated publicly and only available on permission of the approval of corresponding author.

Competing interests

I declare that the aythors have no competing interests or other interests might be perceived to influece the results and/or disccusion reperted in the paper.

Funding

Authors' Contribution:

Study design A

Data Collection B

Statistical Analysis C

Data Interpretation D

Manuscript Preparation E

Literature Search F

Funds Collection G

HH ABCDEF

WL BCD

YW BD

HZ F

ZW DF

MR D

YT AG

Acknowledgements

References

1. Adedara, I.A., Awogbindin, I.O., Anamelechi, J.P., Farombi, E.O., 2015. Garcinia kola seed ameliorates renal, hepatic, and testicular oxidative damage in streptozotocin-induced diabetic rats. Pharm Biol 53, 695-704.

2. Calvani, M., Rapisarda, A., Uranchimeg, B., Shoemaker, R.H., Melillo, G., 2006. Hypoxic induction of an HIF-1alpha-dependent bFGF autocrine loop drives angiogenesis in human endothelial cells. Blood 107, 2705-2712.

3. Cao, Y., Lu, L., Liu, M., Li, X.C., Sun, R.R., Zheng, Y., Zhang, P.Y., 2014. Impact of epigenetics in the management of cardiovascular disease: a review. Eur Rev Med Pharmacol Sci 18, 3097-3104.

4. Chen, J., Somanath, P.R., Razorenova, O., Chen, W.S., Hay, N., Bornstein, P., Byzova, T.V., 2005. Akt1 regulates pathological angiogenesis, vascular maturation and permeability in vivo. Nat Med 11, 1188-1196.

5. Cheng, Y., Jiang, S., Hu, R., Lv, L., 2013. Potential mechanism for endothelial progenitor cell therapy in acute myocardial infarction: Activation of VEGF- PI3K/Akte-NOS pathway. Ann Clin Lab Sci 43, 395-401.

6. Fan, Z., Xu, Z., Niu, H., Sui, Y., Li, H., Ma, J., Guan, J., 2019. Spatiotemporal delivery of basic fibroblast growth factor to directly and simultaneously attenuate cardiac fibrosis and promote cardiac tissue vascularization following myocardial infarction. J Control Release.

7. Ferrara, N., Gerber, H.P., LeCouter, J., 2003. The biology of VEGF and its receptors. Nat Med 9, 669-676.

8. Ferraro, B., Leoni, G., Hinkel, R., Ormanns, S., Paulin, N., Ortega-Gomez, A., Viola, J.R., de Jong, R., Bongiovanni, D., Bozoglu, T., Maas, S.L., D'Amico, M., Kessler, T., Zeller, T., Hristov, M., Reutelingsperger, C., Sager, H.B., Doring, Y., Nahrendorf, M., Kupatt, C., Soehnlein, O., 2019. Pro-Angiogenic Macrophage Phenotype to Promote Myocardial Repair. J Am Coll Cardiol 73, 2990-3002.

9. Frangogiannis, N.G., 2006. The mechanistic basis of infarct healing. Antioxid Redox Signal 8, 1907-1939.

10. Frangogiannis, N.G., 2012. Regulation of the inflammatory response in cardiac repair. Circ Res 110, 159-173.

11. Giacca, M., Zacchigna, S., 2012. VEGF gene therapy: therapeutic angiogenesis in the clinic and beyond. Gene Ther 19, 622-629.

12. Jin, F., Hagemann, N., Sun, L., Wu, J., Doeppner, T.R., Dai, Y., Hermann, D.M., 2018. High-density lipoprotein (HDL) promotes angiogenesis via S1P3-dependent VEGFR2 activation. Angiogenesis 21, 381-394.

13. Kenne Michel, T., Arua Ottoh, A., Christopher Emeka Chukwunonye, U., Christopher Obodoike, E., Christopher, O., Maurice Mmaduakolam, I., 2015. Bio-flavonoids and Garcinoic Acid from Garcinia kola seeds with Promising Anti-Inflammatory Potentials. Pharmacognosy Journal 8, 56-58.

14. Leucker, T.M., Bienengraeber, M., Muravyeva, M., Baotic, I., Weihrauch, D., Brzezinska, A.K., Warltier, D.C., Kersten, J.R., Pratt, P.F., Jr., 2011. Endothelial-cardiomyocyte crosstalk enhances pharmacological cardioprotection. J Mol Cell Cardiol 51, 803-811.

15. Liehn, E.A., Postea, O., Curaj, A., Marx, N., 2011. Repair after myocardial infarction, between fantasy and reality: the role of chemokines. J Am Coll Cardiol 58, 2357-2362.

16. Nahrendorf, M., Swirski, F.K., Aikawa, E., Stangenberg, L., Wurdinger, T., Figueiredo, J.L., Libby, P., Weissleder, R., Pittet, M.J., 2007. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med 204, 3037-3047.

17. Oyagbemi, A.A., Bester, D., Esterhuyse, J., Farombi, E.O., 2018. Kolaviron and Garcinia kola Seed Extract Protect Against Ischaemia/Reperfusion Injury on Isolated Rat Heart. Drug Res (Stuttg) 68, 286-295.

18. Oyagbemi, A.A., Omobowale, T.O., Olopade, J.O., Farombi, E.O., 2017. Kolaviron and Garcinia kola attenuate doxorubicin-induced cardiotoxicity in Wistar rats. J Complement Integr Med 15.

19. Pugh, C.W., Ratcliffe, P.J., 2003. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med 9, 677-684.

20. Rengo, G., Cannavo, A., Liccardo, D., Zincarelli, C., de Lucia, C., Pagano, G., Komici, K., Parisi, V., Scala, O., Agresta, A., Rapacciuolo, A., Perrone Filardi, P., Ferrara, N., Koch, W.J., Trimarco, B., Femminella, G.D., Leosco, D., 2013. Vascular endothelial growth factor blockade prevents the beneficial effects of beta-blocker therapy on cardiac function, angiogenesis, and remodeling in heart failure. Circ Heart Fail 6, 1259-1267.

21. Rengo, G., Zincarelli, C., Femminella, G.D., Liccardo, D., Pagano, G., de Lucia, C., Altobelli, G.G., Cimini, V., Ruggiero, D., Perrone-Filardi, P., Gao, E., Ferrara, N., Lymperopoulos, A., Koch, W.J., Leosco, D., 2012. Myocardial beta(2) -adrenoceptor gene delivery promotes coordinated cardiac adaptive remodelling and angiogenesis in heart failure. Br J Pharmacol 166, 2348-2361.

22. Shah, A.M., Mann, D.L., 2011. In search of new therapeutic targets and strategies for heart failure: recent advances in basic science. Lancet 378, 704-712.

23. Shiojima, I., Sato, K., Izumiya, Y., Schiekofer, S., Ito, M., Liao, R., Colucci, W.S., Walsh, K., 2005. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. J Clin Invest 115, 2108-2118.

24. Tang, J., Shen, Y., Chen, G., Wan, Q., Wang, K., Zhang, J., Qin, J., Liu, G., Zuo, S., Tao, B., Yu, Y., Wang, J., Lazarus, M., Yu, Y., 2017. Activation of E-prostanoid 3 receptor in macrophages facilitates cardiac healing after myocardial infarction. Nat Commun 8, 14656.

25. Tongers, J., Losordo, D.W., Landmesser, U., 2011. Stem and progenitor cell-based therapy in ischaemic heart disease: promise, uncertainties, and challenges. Eur Heart J 32, 1197-1206.

26. Vannella, K.M., Wynn, T.A., 2017. Mechanisms of Organ Injury and Repair by Macrophages. Annu Rev Physiol 79, 593-617.

27. Wallert, M., Bauer, J., Kluge, S., Schmolz, L., Chen, Y.C., Ziegler, M., Searle, A.K., Maxones, A., Schubert, M., Thurmer, M., Pein, H., Koeberle, A., Werz, O., Birringer, M., Peter, K., Lorkowski, S., 2019. The vitamin

E derivative garcinoic acid from Garcinia kola nut seeds attenuates the inflammatory response. Redox Biol 24, 101166.

28. Wang, G.L., Jiang, B.H., Rue, E.A., Semenza, G.L., 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 92, 5510-5514.

29. Winegrad, S., Henrion, D., Rappaport, L., Samuel, J.L., 1998. Vascular endothelial cell-cardiac myocyte crosstalk in achieving a balance between energy supply and energy use. Adv Exp Med Biol 453, 507-514.

30. Yellon, D.M., Hausenloy, D.J., 2007. Myocardial reperfusion injury. N Engl J Med 357, 1121-1135.

31. Yuan, M.J., Wang, T., Kong, B., Wang, X., Huang, C.X., Wang, D., 2016a. GHSR-1a is a novel pro-angiogenic and anti-remodeling target in rats after myocardial infarction. Eur J Pharmacol 788, 218-225.

32. Yuan, M.J., Wang, T., Kong, B., Wang, X., Huang, C.X., Wang, D., 2016b. GHSR-1a is a novel pro-angiogenic and anti-remodeling target in rats after myocardial infarction. Eur J Pharmacol 788, 218-225.

Figures
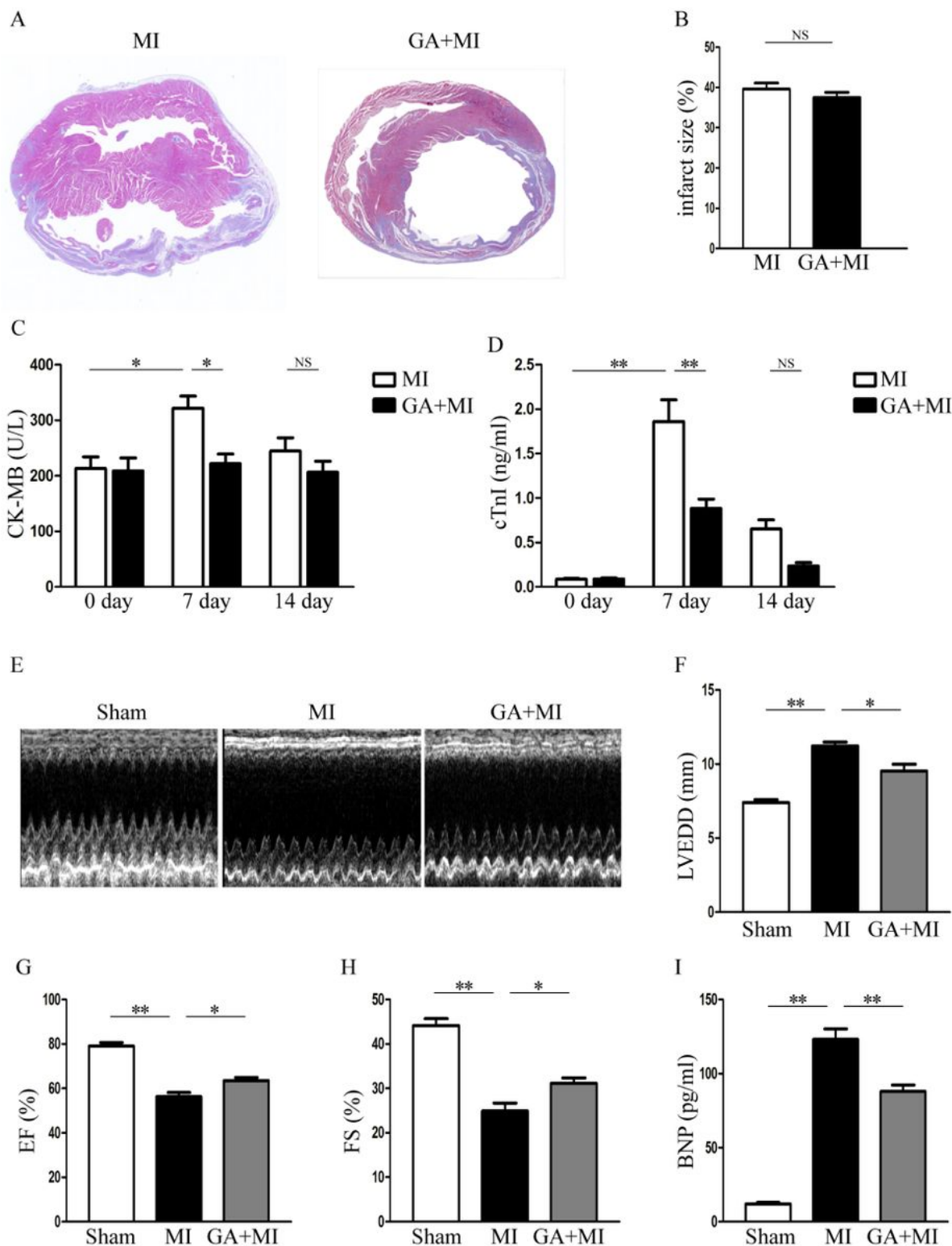
Figure 1

Effects of GA supplement on cardiac function following MI. (A) The representative Masson's trichrome stained myocardial cross-sections at 2 weeks after MI with the treatment of vehicle (MI group) and GA (1 mg/kg, weekly). (B) Quantification of infarcted size. (C, D) The plasma levels of CK-MB and cTnI for rats were measured at 0, 7 and 14 day post-MI by Elisa detection. (E)Representative M-mode echocardiography images. (F-H) Echocardiographic analysis of rats with or without GA supplement at 2

weeks after the MI surgery. LVEDD, Left ventricular end-diastolic diameter; EF, ejection fraction; FS, fractional shortening. (I) Serum levels of BNP by Elisa detection. Values represent the mean ± SEM (n=7). Compared with the matched control, *P <0.05, **< 0.01.
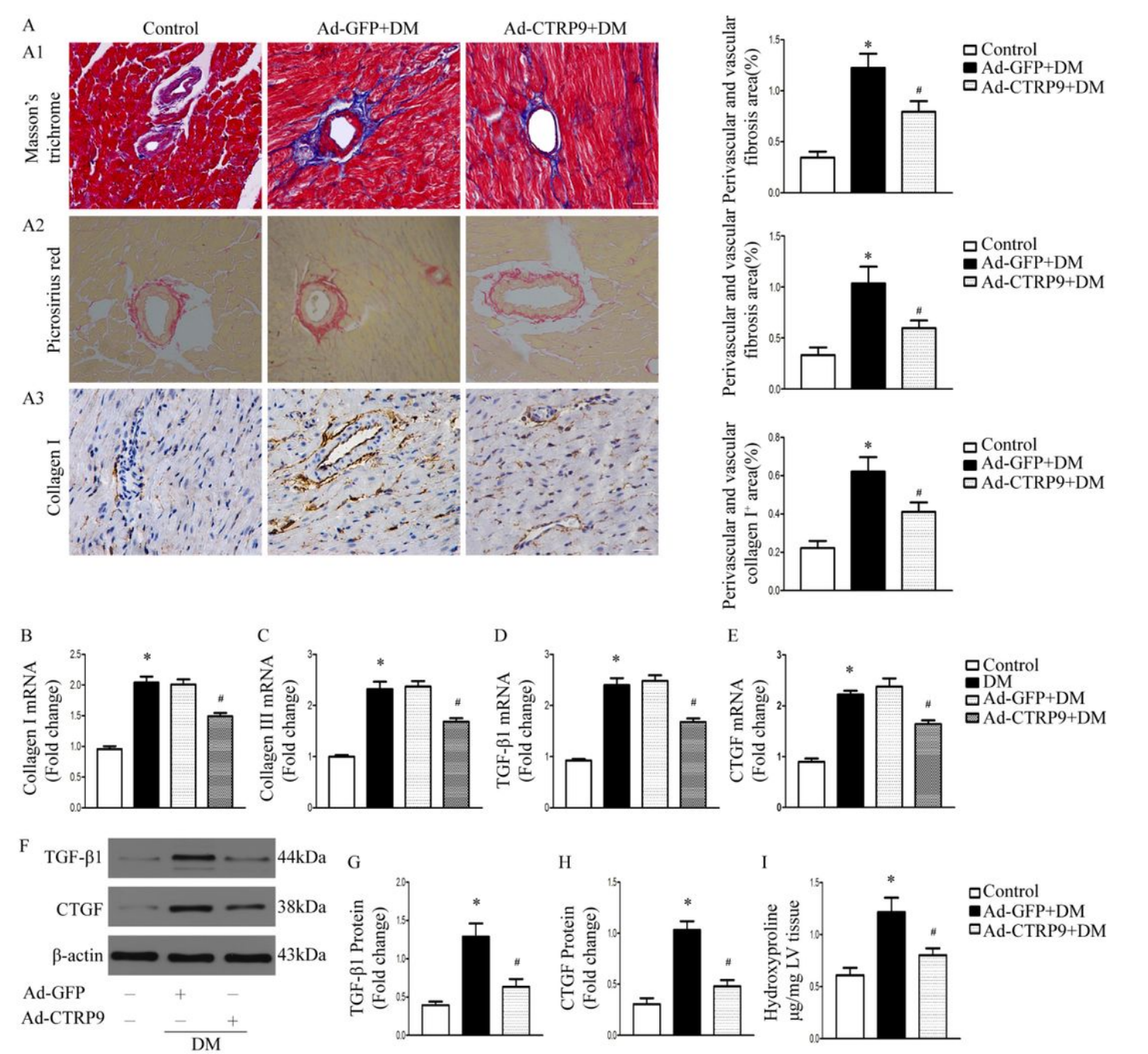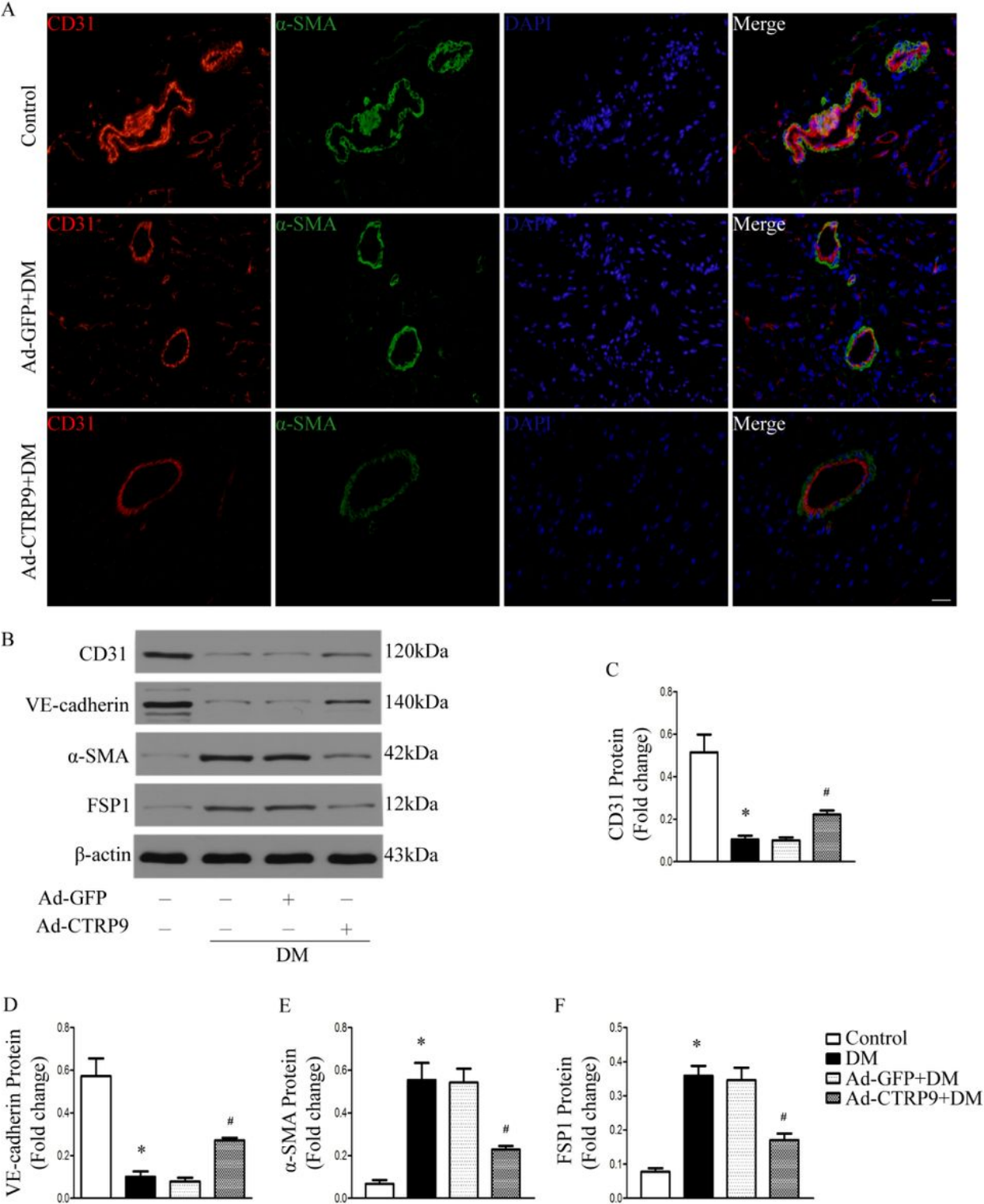

Figure 2

Effects of GA therapy on cardiac angiogenesis. (A, B) Representative images showed double-immunostaining for CD31 (green), α-SMA/Ki67 (red) and DAPI (blue), α-SMA+/ CD31+ (yellow arrows)

expression reflects vessel formation and maturation, Ki67+ (white arrows)/CD31+ (double positive, red arrows) expression represents endothelial proliferation (bar = 50 µm) , inset represents a 16 × zoom. (C-F) Statistical results for positive and co-positive cells in peri-infarcted areas of hearts. Values represent the mean ± SEM (n=4). Compared with the matched control, *P <0.05, **< 0.01, NS indicates not significant.
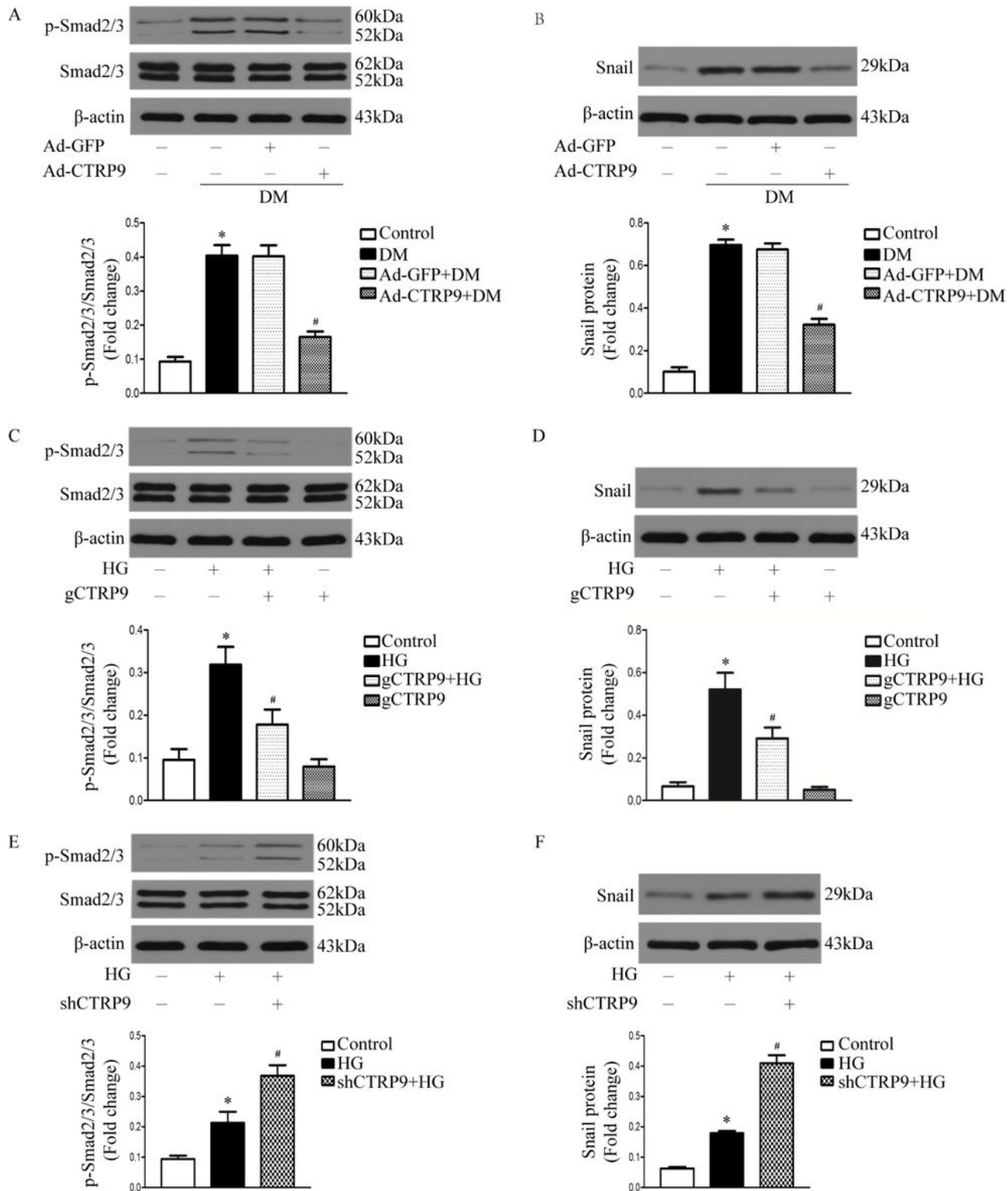

Figure 3

Effects of GA administration on pro-angiogenic proteins and related signaling molecules in vivo study. Representative western blots and quantitative analysis of HIF-1α, VEGF-A and bFGF (A-D), phospho-VEGFR2Tyr1175 (p-VEGFR2 Tyr1175) and VEGFR2 (E-G), and p-Akt, p-eNOS (H-J) from three groups. Values represent the mean ± SEM (n=4). Compared with the matched control, *P <0.05, **< 0.01, NS indicates not significant.
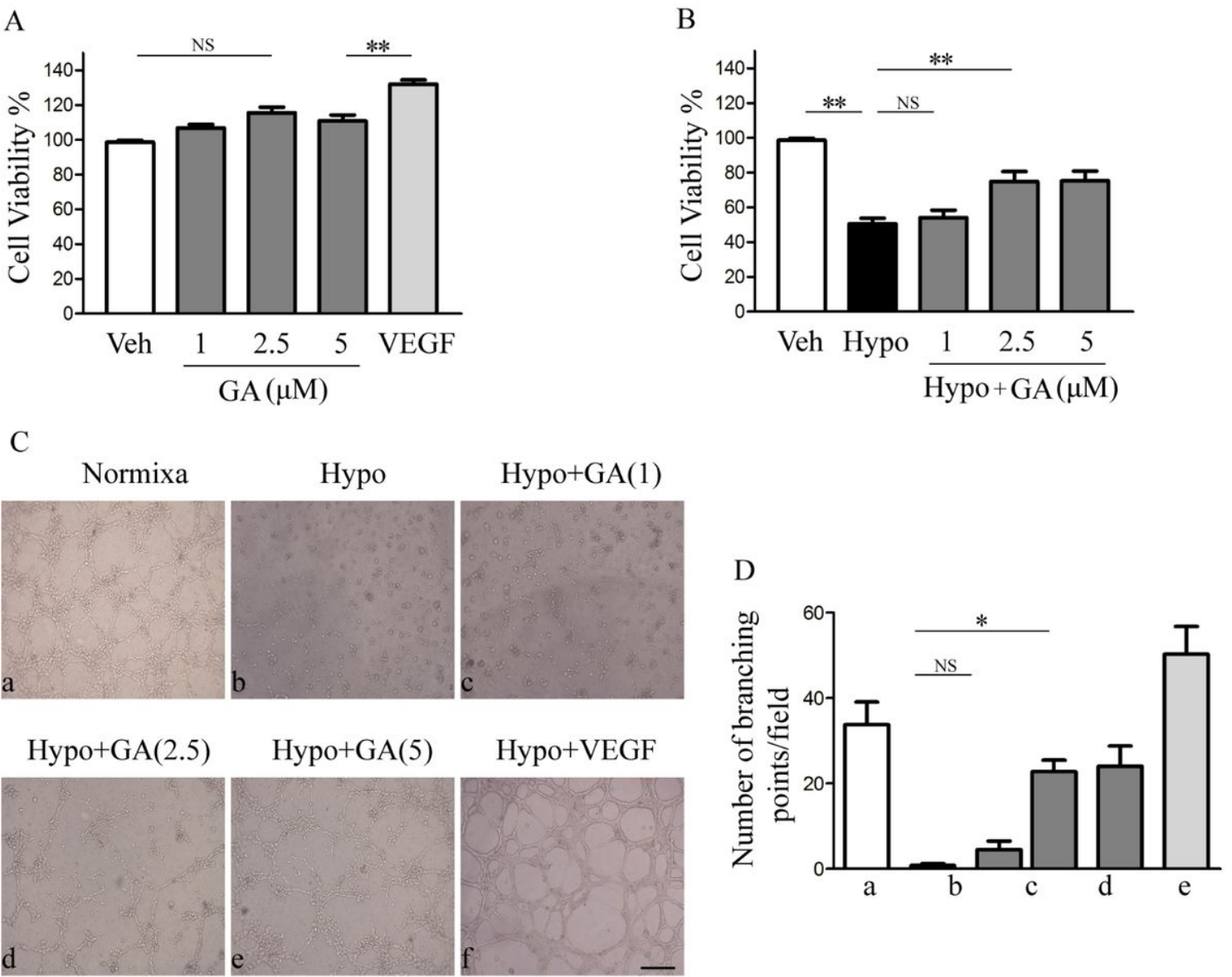
Figure 4

Effects of GA treatment on cell viability and tube formation. Primary cultured HUVECs were pretreated with GA (1, 2.5, 5μM) and VEGF (20 ng/ml) for 30h (A). Pretreatment of different concentrations GA for 24h, and then the cells were exposed to hypoxia for 6 h, cell viability were tested (B). (C-D) HUVECs tube formation was evaluated via using a Matrigel assay. Representative images of vessel-network formation at normixa condition and 6 hours of hypoxia in the presence or absence of GA pretreatment for 24h. hypoxia+VEGF as the control group (bar = 50 μm). Quantifications of tube formation by total branching number. Values represent the mean ± SEM (n=4). Compared with the matched control, *P <0.05, **< 0.01, NS indicates not significant.

Figure 5

Effects of GA treatment on production of pro-angiogenic factors and activities of p-Akt/p-eNOS in HUVECs after hypoxia. Representative western blots and statistical results of HIF-1α, VEGF-A, bFGF in the different does of GA after hypoxia (A-D), p-VEGFR2 Tyr1175 for various durations in normoxia condition and VEGFR2 in hypoxia condition after GA (2.5 μM) pretreatment (E-H), p-Akt and p-eNOS in the

intervention of different doses of GA and hypoxia (I-K). Values represent the mean ± SEM (n=4). Compared with the matched control, *P <0.05, **< 0.01, NS indicates not significant.