RESULTS Repeated tests confirmed concentration impact on the effect of atractylodin acting on VSMCs. The concentration of 5 mg/l and 10 mg/l atractylodin dilutions promote VSMCs proliferation, concentrations greater than 20 mg/l inhibit VSMCs proliferation and exist in dependent relationship between time and concentration. With increasing concentration of atractylodin, cell proliferation inhibition rate was close to 100%. Morphological features of apoptosis were observed by microscope. In the treatment group, many VSMCs were be at the cell cycle G1 phase as indicated by flow cytometry, the quantity of apoptotic cells increased according to time and concentration increases. Western blotting analysis showed, after atractylodin acted on VSMCs, expression of PARP-1, p-IRE1 and GRP78 was significantly higher than the control group, and AIF was activated. However, cleaved-caspase 3, caspase 8 and caspase 9 precursor protein expression remained unchanged. Liquid chip tests confirmed that atractylodin inhibits VSMCs secreted IL-6, IL-8, and TNF-alpha.

CONCLUSIONS Atractylodin may inhibit the proliferation of VSMCs in a dose and time dependent manner. Atractylodin blocks the cell cycle and the cell cycle is arrested in G1 phase. Atractylodin induces caspase-independent apoptosis in VSMCs via PARP-1/AIF apoptotic pathway.

GW27-e0405

Study on the compatibility of Blood Activation and Qi Supplement prescription(BAQS) on angiogenesis regulated by the expression of miR-126 and Spred1 in rats after AMI

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OBJECTIVES To investigate the therapeutic angiogenic effects of BAQS on the expression of vascular endothelial cell specific miR-126 and angiogenic negative regulator Spred1 in ischemic heart disease,and clarify the mechanism and the compatibility of the compound decoction.

METHODS Model rats of acute myocardial infarction (AMI) were established by legation of left anterior descending coronary artery, and randomly divided into 4 groups. Therapeutic groups were treated with BAQS, Blood Activation prescription (BA), or Qi Supplement prescription (QS) and model group was treated with saline. Rats of sham group treated with saline were operated without ligation. Animals were sacrificed and the myocardial infarction border areas were taken as indicators after 4 weeks treatment. Microvascular density (MVD) was assessed by detecting the endothelial specific marker CD31 using Immunohistochemistry. The expression of miR-126 and Spred1 mRNA were detected by quantitative Real-time PCR (qRT-PCR),and Western Blot was used to observe the expression of Spred1.

RESULTS (1)The Immunohistochemistry results of CD31 showed that the MVD in model group was higher than in sham group ($P < 0.01$). Compared with model group, BAQS, BA and QS group can significantly improve MVD ($P < 0.01$). The MVD in BAQS group was higher than in the other two therapeutic groups, but there was no significant difference ($P > 0.05$). (2) The mRNA level of Spred1 in model group was higher than in sham group ($P<0.05$). Compared with model group, BAQ, BA and QS group can significantly improve the mRNA level of Spred1 ($P<0.05$). The mRNA level of Spred1 in BAQS group was lower than in BA group ($P < 0.01$). (3) The expression of Spred1 in model group was higher than in sham group, but there was no significant difference ($P > 0.05$). Compared with model group, BAQS,BA and QS group can significantly reduce the expression of Spred1 ($P < 0.05$). (4) The expression of miR-126 in model group decreased significantly compared with sham group ($P<0.01$). BAQS, BA and QS group can significantly reduced the expression of miR-126 ($P<0.05$). The level of miR-126 in BAQS group was lower than in QS or BA group. However it was only significantly lower than in QS group ($P < 0.01$).

CONCLUSIONS miR-126 reduced and spred1 as well as its mRNA increased abnormally after AMI. To varying degrees, BAQS and its decomposed recipes can promote angiogenesis in myocardial infarction border areas in rats after AMI. The effect of BAQS is the most significant. Its two decomposed recipes work synergistically. The mechanisms of BAQS and its decomposed recipes to promote angiogenesis may be related with their effects on down-regulating the expression of miR-126 and Spred1, up-regulating the mRNA level of Spred1. In addition, we found that the mRNA level of Spred1 increased while the expression of Spred1 decreased. It is suggesting that Spred1 mRNA may be regulated at the post-transcriptional level by other factors, therefore further researches regarding the specific mechanism may be needed.

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Bezafibrate protected against cardiac hypertrophy via inhibiting AKT/GSK-3β and MAPK signaling pathways

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OBJECTIVES Cardiac remolding is characterized as left ventricular dilatation, cardiac fibrosis, and impaired systolic function which predisposes the affected individuals to heart failure. Previous studies had demonstrated thatperoxisome proliferator activated receptor α played key roles in the development of cardiovascular disease by modulate cardiac energy metabolism. Whether BZA could protect pressure overload-induced remodeling has not been completely identified yet.

METHODS The mice were orally given Bezafibrate (BZA) (100mg/kg) from 1 week to 8 weeks after aortic banding (AB). Cardiac function was assessed by echocardiographic parameters including left ventricular diastolic diameter (LVDD), left ventricular ejection fraction (LVEF), and fractional shortening (FS). Body weight (BW), heart weight (HW), and tibia length (TL) were also examined. HE and PSR staining were used to evaluate cross sectional area (CSA) and fibrosis. The hypertrophic and collagen markers were quantitatively determined by RT-PCR. Western blot was applied to detect the expressions of proteins.

RESULTS BZA could decrease LVDD and increase LVEF and FS compared with sham group. Pressure overload resulted in increased HW/BW and HW/TL, while BZA restricted the elevated HW/BW and HW/TL. Decreased CSA and hypertrophic markers were also observed in mice subjected to AB+BZA. BZA also attenuated AB-induced cardiac fibrosis, as illustrated by the decreased collagen volume and fibrotic markers. We also found that the phosphorylation of protein kinase B (AKT)/glycogen synthase kinase-3β (GSK3β) and mitogen-activated protein kinase (MAPK) were significantly downregulated under the BZA treatment.

CONCLUSIONS Our study demonstrated that BZA could alleviate cardiomyocyte hypertrophy and suppress cardiac fibrosis in mice.

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Cathepsin L is responsible for wire injury- induced intimal hyperplasia

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OBJECTIVES Intimal hyperplasia (IH) is characterized by inflammatory cells infiltration and extensive smooth muscle cells proliferation that contributes to restenosis after percutaneous coronary intervention (PCI). Cathepsin L (CatL), a member of lysosome protease is highly expressed in mouse intimal hyperplasia lesions, but whether it contributes to the pathogenesis of IH remains unknown.

METHODS Carotid artery intimal hyperplasia was induced in WT and CatL KO (CatL-/-) mice by wire injured surgery, and IH lesions were analyzed after 28 days. Expression of CatL in the carotid lesions was analyzed by RT-PCR, Immunofluorescent staining and western blot. CatL's activity was measured by CathepsinL Activity Assay Kit (Fluorometric). Migration, proliferation and cytokine (MCP-1) production of bone marrow macrophages or smooth muscle cells derived from WT and CatL-/- mice were investigated. Vascular reactivity was studied in mice aortas.

RESULTS We found that CatL expression and activity increased significantly in mouse IH lesions. The IH, intimal area and intimal/media ratio, in CatL-/- mice was significantly reduced than that in WT mice. CatL deficiency impaired macrophage infiltration 3 days after wire injury surgery, but did not affect smooth muscle cell or endothelial function directly. Proliferation and migration functions of smooth muscle cells derived from WT and CatL-/- aorta showed no significant difference. Aortic rings from WT and CatL-/- mice showed no significant difference in acetylcholine-induced endothelial relaxation responses. In vitro studies proved that production of