RESULTS After Model of EAHy926 cell injured by homocysteine was made, we found that cultured with 0.5, 1.0, 2.0, 4.0, 8.0 μM homocysteine, cells grew less than cultured with normal culture medium, Cells cultured with homocysteine 4.0 μM/L for 24h was made to be the model of injury. To detect the effect of aconine on EAHy926 cell injured by homocysteine, well growing EAHy926 cells were cultured in culture plate. 24h later, cells were cultured with DMEM medium containing 2% fetal calf serum for 8 hours to make cells hungry, then cultured with medium containing aconine 0, 0.05, 0.10, 0.20 mg/ml respectively for 30 minutes, then cultured with medium containing aconine 0.40 μM/L for 24h. It was found that, compared with control group, attached cells in aconine groups grew better, and attached cells in aconine 0.20 mg/ml plus homocysteine 4.0 μM/L group grew best. Detected by nitrate reductase method, it was found that compared with control group, there was no obvious change of cell viability. In aconine groups, cell viability increased in aconine 0.20 mg/ml plus homocysteine 4.0 μM/L group, but in homocysteine 4.0 μM/L medol group, NO concentration of cell culture fluid decreased obviously, and in aconine groups, NO concentration of cell culture fluid increased, and in aconine 0.20mg/ml plus homocysteine 4.0μmol/L group it was the most obvious (p <0.05). Detected by Western-blot, it was found that, compared with control group, there was no obvious change of protein of Sirt-1 and eNOS in aconine 0.20 mg/ml group, but in homocysteine 4.0 μM/L model group, expression of Sirt-1 and eNOS protein weakened obviously, and in aconine groups, expression of Sirt-1 and eNOS protein enhanced, and in aconine 0.20 mg/ml plus homocysteine 4.0 μM/L group it was the most obvious (p <0.05). Detected by fluorescent quantitation, it was found that, compared with control group, there was no obvious change of mRNA of Sirt-1 and eNOS in aconine 0.20 mg/ml group, but in homocysteine 4.0 μM/L model group, expression of Sirt-1 and eNOS mRNA weakened obviously, and in aconine groups, expression of Sirt-1 and eNOS mRNA enhanced, and in aconine 0.20 mg/ml Plus homocysteine 4.0 μM/L group, it was the most obvious (p <0.05).

CONCLUSIONS Homocysteine may injure EAHy926 cell by suppressing the expression of Sirt-1 then suppressing the expression of eNOS system, while aconine may protect EAHy926 cell by enhancing the expression of cavelin-1 then enhancing the expression of eNOS system.

GW26-e5354 Suv39h1 Promotes Neointima Formation in Diabetes
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OBJECTIVES Patients with diabetes are at an increasing risk of vascular complications. Suv39h1, a histone methyltransferase, is suggested to participate in various vascular smooth muscle cells (VSMCs) from the metabolic memory and proinflammatory phenotype in diabetes (DM). Yet the direct effects of Suv39h1 on DM-induced vascular remodeling remain largely elusive.

METHODS In this study, by generating adenovirus expressing Suv39h1 (Ad-Suv39h1) or lentivirus expressing Suv39h1-targeting shRNA (LV-Suv39h1 shRNA), we evaluated the significance of Suv39h1, in VSMCs under diabetic conditions both in vitro and in vivo. In vitro, we examined the proliferative and migratory behavior as well as the underlying signaling molecules of VSMC in response to high glucose (5.5 mmol/L, HG) treatment. In vivo, we induced diabetes in SD rats with intraperitoneal injection of streptozocin and established the common carotid artery balloon injury model. Microarray analysis was performed to identify altered gene profile in carotid arteries after balloon injury.

RESULTS In response to HG, the endogenous Suv39h1 level was significantly reduced by almost 50% in VSMCs. Following HG treatment, Ad-Suv39h1-infected VSMCs exhibited significantly higher proliferative and migratory capabilities than control group. In contrast, knocking down Suv39h1 by LV-Suv39h1 shRNA led to dramatically reduced proliferation and migration. Through analyzing the molecular mechanisms, knocking down Suv39h1 reduced the levels of ID3, complement C3 and phosphor-ERK1/2 in response to HG, but increased the level of p21 and p27KIP1 as compared to control group. Overexpressing Suv39h1 has the opposite effect on the expression of these molecules. Consistent with the in vitro observation, ectopic expression of Suv39h1 was sufficient to up-regulate complement C3 and ID3 (P < 0.05, as compared to Ad-Null), while knocking down Suv39h1 was capable of reducing these two molecules in injured vessels (P < 0.05, as compared to LV-NC shRNA). As we expected, Suv39h1 overexpression led to accelerated neointima formation, while knocking down Suv39h1 reduced it following carotid artery injury in diabetic rats. Finally, altering Suv39h1 level in vivo dramatically changed the expressions of myriad genes with various functions and participating in different biological processes revealed by microarray analysis. We identified 233 up-regulated, 221 down-regulated genes between Ad-Suv39h1 and Ad-Null group, and 549 up-regulated, 658 down-regulated genes between LV-Suv39h1 shRNA and LV-NC shRNA group. Further Gene Ontology (GO) analysis showed that these altered genes participated in diverse biological processes, such as metabolic processes, cell differentiation, cell development, response to external stimulus.

CONCLUSIONS This study reveals the novel functions of Suv39h1 within VSMC in diabetes and suggests its potential role as a therapeutic target for diabetic vascular injury.

GW26-e2216 Role of IKK:γ in high glucose-concentration-induced endothelial cell dysfunction and insulin resistance by IKK:γ gene silencing
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OBJECTIVES The study is to investigate the effects of expression downregulation in IKK:γ on high glucose-induced vascular endothelial insulin resistance (IR) and dysfunction.

METHODS HUVECs were inoculated in a 6-well plate uniformly and were randomly divided into four groups, namely, Control group, HG group, Non-silencing shRNA group, and RNAi group, respectively. Control group were only treated with DMEM containing glucose (5.5 mmol/L) for 48h, while other groups were treated with DMEM containing glucose (22 mmol/L) for 48h and insulin(100 nmol/L) for 30 minutes post transfection. The expression levels of IKK:γ mRNA and protein, cell viability, NO and ET-1 level were detected.

RESULTS HUVECs were incubated in media containing 33mmol/L of glucose for 48h and 100nmol/L of insulin for 30 minutes, the cell viability was significantly decreased, showing significant difference between HG group and Control group (P < 0.01); it was also found that the levels of ET-1 was increased. however, the levels of NO was decreased, which had significant difference compared with Control group (P < 0.05). Downregulation of IKK:γ expression by its gene silencing decreased the expression levels of IKK:γ mRNA and its protein absolutely and may antagonize these changes in all above-mentioned parameters induced by high glucose concentration and insulin, and there was significant difference between RNAi group and HG group (P < 0.05). However, the results of the Non-silencing shRNA group were similar to the High group and there was no significant difference between the two groups (P > 0.05).

CONCLUSIONS The silencing of IKK:γ gene could protect the HUVECs in high glucose-induced vascular endothelial insulin resistance (IR) and dysfunction.

GW26-e2219 Danlou Tablet Decreases Cytokine IFN-γ and Attenuates the Progression of Atherosclerosis
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OBJECTIVES Atherosclerosis is an inflammatory disease in which the Th1 cytokine IFN-γ plays a central role. We investigated whether Danlou Tablet, which promoting blood circulation and eliminating phlegm in Traditional Chinese Medicine, could decrease the expression of IFN-γ and restrain the Th1 cells activated to alleviate atherosclerosis in apolipoprotein E-deficient ApoE-/- mice.

METHODS Eight-week old ApoE-/- mice fed a high-fat diet for 16 weeks, at the mean time were randomly divided into six groups for 16wk. Control group that fed a Chow diet and received no treatment, other five groups fed a high-fat diet, model group that also received no treatment, low-dose DLT group (1 g/kg/d), moderate-dose DLT group (2 g/kg/d), high-dose DLT group (4 g/kg/d) were administered Danlou Tablet suspension, and atorvastatin group that were treated with atorvastatin. Cell suspensions were obtained from the spleens of five randomly chosen mice from each group at the 16 week. The flow cytometry analysis were used to detect the percentages of CD4 + IFN-γ+ (Th1 cells) in the spleen cell suspensions. Immunohistochemical
staining and real-time PCR were used to detect the expression of IFN-γ from the aorta of ApoE−/− mice.

RESULTS  Our results show that the percentages of CD4+ IFN-γ+ from the spleens were decreased after treatment with different dose of Danlou Tablet groups compared to the model group, greatly decreased with Danlou Tablet dose-dependent (the fold changes of IFN-γ in model group vs DLT-H group: 13.80±2.00 vs 8.23±1.65, p < 0.01). Immunohistochemical staining and real-time PCR for aorta revealed that the IFN-γ was decreased with Danlou Tablet dose-dependent (the fold changes of IFN-γ in model group vs DLT-L group: 2.96±0.87 vs 2.10±1.28, P < 0.05; model group vs DLT-M group: 2.96±0.87 vs 2.45±0.05, P < 0.01). The findings suggest that Danlou Tablet could markedly attenuates progression of atherosclerosis by decreasing the expression of IFN-γ and suppressing inflammation.

CONCLUSIONS  Our findings demonstrate that Danlou Tablet could decrease the expression of IFN-γ or retrain the artery-infiltrating T cells, especially the Th1 cells activated or suppressed inflammation and attenuated progression of atherosclerosis via weaken the Th1-IFN-γ pathway.

GW26-e2323 Regulation and mechanism of Guizhi Decoction on Diabetic Cardiac Autonomic Neuropathy

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OBJECTIVES  To investigate the effects of Guizhi decoction (a long-used harmonic prescription in traditional Chinese medicine) on cardiac autonomic neuropathy (CAN) in streptozotocin (STZ)-induced diabetic rats. With the establishment of chemical sympathetic sprouting and sympathetic ratemyopathy models, we explored the protective mechanism of Guizhi decoction on the cardiac sympathetic remodeling.

METHODS  1) After induction of diabetic rats with STZ for four weeks, mecabolamin and Guizhi decoction were administered to the STZ rats for 4 weeks. Heart rate variability (HRV) were recorded, and contents of nerve growth factor (NGF), growth associated protein 43 (GAP-43) and ciliary neurotrophic factor (CNTF) in myocardium as well as the density of tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) in the right atrium (RA) were respectively measured using ELISA and immunohistochemical staining.

2) Cardiac sympathetic sprouting rats were induced with 4-Methyl catechol (4-MC) for 30 days. Metoprolol and Guizhi decoctions were separately administered to the rats for 30 days. Meanwhile, we established chemical sympathopathy rats with 6-Hydroxydopamine (6-OHDA) for 3 days. Mecobalamin and Guizhi decoctions were separately administered to the rats for 10 days. Contents of GAP-43, TH, ChAT, TGFβ, IL-6 and IL-1β in the cardiac tissue were examined and distributed into TH-positive nerve fibers was observed. Besides, serum myocardial enzyme levels and changes of myocardial morphology were observed in 6-OHDA rats.

RESULTS  1) STZ rats demonstrated autonomic nerve dysfunction. Compared with the model groups, rats treated with Guizhi decoction had higher HRV parameters such as SDNN, RMSSD, LF, HF and TP, as well as decreased LF/HF (P < 0.05); the TH-positive nerve fibers decreased while the ChAT-positive expression increased (P < 0.05); contents of GAP-43 and CNTF increased while NGF decreased (P < 0.05). It suggested that Guizhi decoction improved vagal nerve dysfunction in STZ diabetic rats and mitigated the autonomic neuropathy.

2) 4-MC caused cardiac sympathetic sprouting. Compared with the model group, contents of TH and GAP-43 in cardiac tissue decreased while ChAT unchanged in Guizhi decoction group, indicating that Guizhi decoction can effectively suppress the 4-MC-induced sympathicopathy.

3) 6-OHDA caused chemical sympathopathy. Compared with the model group, contents of TH and GAP-43 in myocardium of Guizhi decoction group increased and ChAT kept unchanged; the serum levels of myocardial enzymes, the cardiac histopathology and heart function were improved, suggesting that Guizhi decoction effectively alleviated sympathetic injury and myocardial injury related to sympathetic denervation.

CONCLUSIONS  Guizhi decoction effectively alleviated diabetic CAN. Guizhi decoction demonstrated double-sided regulation on cardiac sympathetic sprouting and sympathetic denervation which may be one of the important mechanism of the improvement of Guizhi Decoctionon on diabetic cardiomyopathy.

GW26-e4511 Blockade of Histone Demethylase JMJD2A Stimulates Endothelial Repair in Denuded Aorta of diabetic Rat

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OBJECTIVES  Endothelial dysfunction is a central hallmark of diabetes, delaying endothelial healing in diabetic patients following angioplasty. The epigenetic abnormalities are increasingly considered to be relevant to the pathogenesis of diabetic complications. We here investigated the therapeutic potential of histone demethylase JMJD2A and its substrate histone H3 lysine 9 trimethylation (H3K9me3) on re-endothelialization in a vascular injury model of diabetic rats.

METHODS  JMJD2A inhibition was achieved either by chemical inhibition (2,4-pyridindicarboxylic acid (2,4-PDCA) or small interfering RNA (siRNA) both in vitro and in vivo studies. In vitro, we examined the proliferative, migratory and angiogenic capacities of human umbilical vein endothelial cells (HUVECs) in response to high glucose (HG). The Endothelial function was determined to detect the apoptosis and the expressions of apoptosis-associated genes were assessed by real-time PCR and western blot. Immunoprecipitation (ChIP) assay was conducted to examine the modification of H3K9me3 at related genes’ promoters. In vivo, common carotid artery balloon injury was developed and its effects were significantly increased in high-fat diet (60% fat) and low-dose streptozotcin (35 mg/kg) induced diabetic rats. The reendothelialization was quantified with Evans blue staining and immunohistochemical staining.

RESULTS  Both in HG-treated HUVECs and balloon-injured arteries of diabetic rats, the global expression of JMJD2A was increased whereas H3K9me3 was decreased. In vitro, JMJD2A inhibition either by 2,4-PDCA (0.5mM) or by siRNA (20nM) accelerated HUVECs proliferation, migration and tube formation in response to HG, accompanied by reduced expression of TNF-a and suppressed apoptosis (down-regulated caspase3, caspase9, Bax and upregulated Bcl-2). ChIP assay indicated that the potential mechanism was relevant to the increased H3K9me3 at the promoter of TNF-a and then the transcriptional silencing of TNF-a. Complementary in vitro studies showed that JMJD2A inhibition promoted reendothelialization in diabetic rats.

CONCLUSIONS  JMJD2A inhibition promotes reendothelialization after arterial injury in diabetic rats via accelerated proliferation, migration and suppressed apoptosis of endothelial cells.

GW26-e5383 Pioglitazone Decreases Plate Thrombosis by Attenuating Platelet Inflammation. An In Vivo Study Using 18F-FDG PET/CT

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OBJECTIVES  Pioglitazone, a clinically used insulin sensitizer, has appeared to have an anti-atherosclerotic effect. The aims of this study is to determine whether pioglitazone can reduce the number of plaque thrombosis incidences and whether decreasing plaque inflammation is the mechanism by which pioglitazone reduces plaque thromboses in an experimental atherosclerotic model.

METHODS  20 male New Zealand white rabbits were randomly divided into two groups: Atherosclerosis group (Group A, n=10) and middle-pioglitazone-treated group (Group P, n=10). Atherosclerosis was induced in all rabbits by intermittent high-cholesterol diet and endothelial denudation. From the ninth week, the rabbits in group P received pioglitazone (10 mg kg−1 d−1) in addition to the diet, till the end of experiment. PET/CT scans were performed at 8 week and 18 week in all survival rabbits, to obtain FDG uptake parameters (mean standardized uptake value, SUVmean and maximal standardized up- take value, SUVmax). Concomitantly, serum samples were obtained for analysis of blood glucose (G), triglycerides (TG), total cholesterol (Ch), HDL, LDL, hs-CRP and matrix metalloproteinase-9 concentration (MMP-9). All survival rabbits underwent 2 pharmacological triggerings to induce plaque rupture at 18 week. After pharmacological triggering, all rabbits were euthanatized, aortic histopathological analysis were performed.

RESULTS  20 male New Zealand white rabbits were randomly divided into two groups: Atherosclerosis group (Group A, n=10) and