# 冠心宁片减轻ox-LDL损伤内皮细胞的药效物质研究

余 敏[1], 王木兰[2], 赵 璐[1], 王 毅[1,3*]

(1. 浙江大学药学院, 浙江 杭州 310058; 2. 正大青春宝药业有限公司, 浙江 杭州 310023; 3. 浙江大学智能创新药物研究院, 浙江 杭州 310018)

**摘要**: 本文研究冠心宁片对氧化低密度脂蛋白 (oxidized low density lipoprotein, ox-LDL) 诱导人脐静脉内皮细胞 (human umbilical vein endothelial cells, HUVECs) 损伤的干预作用, 为冠心宁片治疗动脉粥样硬化提供实验依据。在ox-LDL对HUVECs细胞的损伤下, 采用CCK-8 (cell counting kit-8) 法检测细胞的活力; 采用相应的试剂盒检测细胞培养上清中的乳酸脱氢酶 (lactate dehydrogenase, LDH) 含量; 用普通相差显微镜观察不同组别的细胞形态; 采用DCFH-DA和DAF-FM DA探针分别检测细胞中的活性氧 (reactive oxygen species, ROS) 和NO水平; 单核细胞黏附实验检测HUVECs对THP-1的招募情况; 用TMRM染料检测线粒体膜电位水平; ELISA法检测细胞的白细胞介素-6 (interleukin-6, IL-6)、细胞间黏附因子-1 (intercellular adhesion molecule-1, ICAM-1) 和单核细胞趋化蛋白-1 (monocyte chemoattractant protein-1, MCP-1) 分泌情况。结果显示, 冠心宁片对HUVECs具有浓度依赖性促增殖作用, 在100 μg·mL$^{-1}$ ox-LDL刺激下, 内皮细胞形态发生明显改变, 此时NO水平显著下降, ROS水平显著上升并伴随着线粒体膜电位下降, 内皮细胞对THP-1细胞的招募增加, IL-6、ICAM-1和MCP-1也显著升高, 产生了氧化应激和炎性损伤。而冠心宁片及其组成的丹参、川芎提取物能够明显改善细胞形态、提高NO水平、降低ROS的产生, 并且还能减少炎症相关蛋白IL-6和MCP-1的分泌。在NO这个内皮基础功能的指标上, 丹参和川芎具有显著的协同作用。其中丹酚酸B和丹参素发挥主要的药效作用, 丹参素和阿魏酸联合药效优于单给药药效。以上结果表明, 冠心宁片及其药效物质具有改善内皮基础功能、抵抗氧化应激、缓解炎性损伤的作用, 并且丹参和川芎协同增效, 可能与其调控氧化应激和炎症有关, 在动脉粥样硬化的防治中具有应用前景。

**关键词**: 冠心宁片; 丹参; 川芎; 动脉粥样硬化; 协同作用; 氧化低密度脂蛋白; 炎症; 氧化应激

**中图分类号**: R966 **文献标识码**: A **文章编号**: 0513-4870(2023)01-0139-10

# Guanxinning Tablet and their active substances alleviate ox-LDL-induced endothelial cell injury

YU Min[1], WANG Mu-lan[2], ZHAO Lu[1], WANG Yi[1,3*]

(*1. College of Pharmaceutical Science, Zhejiang University, Hangzhou 310058, China; 2. Chiatai Qingchunbao Pharmaceutical Co., Ltd., Hangzhou 310023, China; 3. Innovative Institute for Artificial Intelligence in Medicine of Zhejiang University, Hangzhou 310018, China*)

**Abstract**: This study investigated the intervention effect of Guanxinning Tablet on human umbilical vein endothelial cells (HUVECs) injury induced by oxidized low density lipoprotein (ox-LDL), providing experimental basis for Guanxinning Tablet in the treatment of atherosclerosis-related diseases. Under the damage of HUVECs by ox-LDL, the cell viability was detected by CCK-8 (cell counting kit-8) assay; lactate dehydrogenase (LDH) in the cell culture supernatant was detected by the corresponding kit; the cell morphology of different groups was

收稿日期: 2022-05-30; 修回日期: 2022-07-24.
基金项目: 国家自然科学基金资助项目 (82173941).
*通讯作者 Tel: 86-571-88208426, E-mail: mysky@zju.edu.cn
DOI: 10.16438/j.0513-4870.2022-0663

observed by common phase contrast microscope; reactive oxygen species (ROS) and NO levels in the cells were detected by DCFH-DA and DAF-FM DA probes, respectively; monocyte adhesion assay was used to detect the recruitment of THP-1 in HUVECs, and TMRM dye was used to detect the level of mitochondrial membrane potential; interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) secretion in the cells was detected by ELISA assay. The results showed that Guanxinning Tablet had a concentration-dependent proliferative effect on HUVECs. Under the stimulation of 100 μg·mL$^{-1}$ ox-LDL, the morphology of endothelial cells was significantly changed. At this time, NO level was significantly decreased, ROS level was significantly increased and accompanied by a decrease in mitochondrial membrane potential. The recruitment of THP-1 cells by endothelial cells and IL-6, ICAM-1 and MCP-1 were also significantly increased, resulting in oxidative stress and inflammatory injury. Guanxinning Tablet and its composed extracts could significantly improve cell morphology, increase NO level, decrease ROS production, and also reduce the secretion of inflammation-related proteins IL-6 and MCP-1. *Salvia miltiorrhiza* and *Ligusticum striatum* DC. have significant synergistic effects on NO. Among them, salvianolic acid B and salvianic acid A exerted the main effects, and the combined efficacy of salvianic acid A and ferulic acid was superior to that of single administration. The above results showed that Guanxinning Tablet and their active substances had the effects of improving endothelial basal function, resisting oxidative stress, and alleviating inflammatory injury, and *Salvia miltiorrhiza* and *Ligusticum striatum* DC. synergized, which may be related to their regulation of oxidative stress and inflammation and have application prospects in the treatment of atherosclerosis-related diseases.

**Key words**: Guanxinning Tablet; *Salvia miltiorrhiza*; *Ligusticum striatum* DC.; atherosclerosis; synergistic effect; oxidized low density lipoprotein; inflammation; oxidative stress

动脉粥样硬化 (atherosclerosis, AS) 是一种由脂质代谢紊乱和慢性炎症等多因素诱导的血管性疾病, 动脉内膜呈黄色粥样改变, 是心脏病、脑卒中等心脑血管疾病的主要诱发因素[1-3]。尽管AS的发病机制尚未完全阐明, 但内皮功能障碍被公认为是AS发病和发展的驱动力[4]。氧化理论认为, 高胆固醇血症触发氧化低密度脂蛋白 (oxidized low density lipoprotein, ox-LDL) 在内膜下间隙蓄积, 是AS的主要风险因素[2,3]。ox-LDL损伤内皮细胞, 诱导炎症性因子的释放, 包括几种趋化因子和细胞表面黏附分子, 如单核细胞趋化蛋白-1 (monocyte chemoattractant protein-1, MCP-1)、E-选择素 (E-selectin)、细胞间黏附因子-1 (intercellular adhesion molecule-1, ICAM-1)、血管细胞黏附因子-1 (vascular cell adhesion factor-1, VCAM-1) 等, 黏附分子的表达升高促进炎性单核细胞或白细胞黏附于内皮, 巨噬细胞吞噬大量脂质形成泡沫细胞, 并促进平滑肌细胞的迁移和增殖[5-7]。除了刺激内皮细胞产生炎症, ox-LDL还触发氧化应激和细胞凋亡[8]。凝集素样ox-LDL受体-1 (lectin-like ox-LDL receptor-1, LOX-1) 是内皮细胞上的特异性受体, 也是内皮功能障碍的主要标志蛋白[9]。除此之外, ox-LDL还通过多种不同的机制损伤内皮细胞, 如丝裂原活化蛋白激酶 (mitogen-activated protein kinase, MAPK) 和核因子-κB (nuclear factor-κB, NF-κB) 信号通路等[6]。

冠心宁片 (Guanxinning Tablet, GXN) 由临床上应用多年的冠心宁注射液改良剂型转化而来, 是2015年经国家批准上市的治疗心血管类疾病的中成药[10], 具有活血化瘀、通脉养心的功效[11]。冠心宁片由丹参和川芎两种草药以1∶1的质量比率混合, 然后经过水提、混合、浓缩、醇沉、过滤、再浓缩、真空干燥等程序制备而成[12]。丹参味苦, 性微寒, 行活血化瘀、镇静安神之效, 为君药; 川芎味辛, 性温, 有活血行气、祛风止痛之功, 为使药, 两药君使配伍[10,13]。有研究报道丹参饮具有干预和治疗动脉粥样硬化的新用途[14], 川芎被报道过有抗炎活性[15]。在活血化瘀中成药配伍中, 丹参和川芎是最常用的药对[16]。目前冠心宁片已有多种明确的药效, 包括抗心肌缺血再灌注损伤[17-19]、抗血栓形成[12,20-22]、促进血管生成[23]、扩血管作用[24]和抗心力衰竭[11]等作用。尽管冠心宁片已有许多治疗心血管疾病的报道, 但是对于AS起始阶段关键环节——内皮损伤所发挥的影响却研究得相对较少, 且药效物质不够明确。已有研究表明冠心宁注射液能够保护叔丁基过氧化氢所引起的内皮损伤[25]。本研究通过构建ox-LDL损伤HUVECs的细胞模型, 在氧化应激和炎性损伤两方面探究冠心宁片及其药效物质对内皮的保护作用, 为冠心宁片治疗动脉粥样硬化提供实验依据。

## 材料与方法

**试剂** 丹酚酸B (salvianolic acid B, SB, 批号B20261, CAS: 115939-25-8)、丹参素 (salvianic acid A,

SA, 批号B20254, CAS: 76822-21-4)、迷迭香酸 (rosmarinic acid, RA, 批号B20862, CAS: 20283-92-5)、阿魏酸 (ferulic acid, FA, 批号B20007, CAS: 1135-24-6) 和洋川芎内酯I (senkyunolide I, SI, 批号B21463, CAS: 94596-28-8) (上海源叶生物科技有限公司); 胎牛血清、青霉素溶液 (10 000 u·mL$^{-1}$) 和链霉素溶液 (10 000 μg·mL$^{-1}$) (Gibco公司); DMEM高糖培养基 (Corning公司); 活性氧 (ROS) 探针 (DCFH-DA)、四甲基罗丹明甲酯 (TMRM) (Sigma-Aldrich公司); 乳酸脱氢酶 (LDH) 试剂盒、一氧化氮 (NO) 荧光探针 (DAF-FM DA)、细胞质染料Calcein-AM (碧云天生物技术有限公司); 细胞核染料Hoechst 33342 (Invitrogen公司); ELISA试剂 (义翘神州科技股份有限公司); CCK8试剂 (美仑生物技术有限公司); 磷酸盐缓冲液 (PBS, 凯基生物技术有限公司)。

**仪器**　二氧化碳细胞培养箱 (Thermo Fisher公司); Infinite M1000Pro多功能酶标仪 (TECAN公司); 倒置相差显微镜 (Leica公司); Image Xpress Pico自动细胞成像及分析系统、Image Xpress Micro Confocal高内涵成像系统 (Molecular Devices公司)。

**冠心宁片浸膏的制备**　冠心宁片以及丹参提取物 (*Salvia miltiorrhiza* Bunge, DS) 和川芎提取物 (*Ligusticum striatum* DC., CX) 由正大青春宝药业有限公司生产 (批号: 201912), 依照中国国家食品药品监督管理局标准 (YBZ00342016) 执行。简言之, 丹参和川芎两种草药以1∶1的质量比率混合, 然后经过水提、混合、浓缩、醇沉、过滤、再浓缩、真空干燥等程序制备成的干燥粉末即为冠心宁片。在本实验批次的冠心宁片中, 1 g冠心宁片来源于6.46 g的草药 (3.23 g丹参草药和3.23 g川芎草药), 而1 g丹参提取物来源于10.36 g丹参草药, 1 g川芎提取物来源于6.97 g川芎草药, 即1 g冠心宁片相当于0.311 8 g丹参提取物与0.445 2 g川芎提取物之和。

**细胞培养**　HUVECs和THP-1细胞培养在含10%胎牛血清、100 u·mL$^{-1}$青霉素和100 μg·mL$^{-1}$链霉素的DMEM高糖培养基中, 置于37 ℃、5% $CO_2$的细胞培养箱中。细胞每3天传代, 处于对数生长期时用于实验。

**细胞模型及分组**　取对数生长期的HUVECs细胞消化后, 以8 000个/孔的密度接种于96孔细胞培养板中, 贴壁生长24 h。实验如下分组: 对照组 (DMEM)、模型组 (100 μg·mL$^{-1}$ ox-LDL)、冠心宁组 (400 μg·mL$^{-1}$ GXN + 100 μg·mL$^{-1}$ ox-LDL)、丹参组 (124.8 μg·mL$^{-1}$ DS + 100 μg·mL$^{-1}$ ox-LDL) 和川芎组 (185.6 μg·mL$^{-1}$ CX + 100 μg·mL$^{-1}$ ox-LDL)。

**CCK-8法检测细胞存活率**　用不含血清的培养基将冠心宁配成不同浓度 (12.5、25、50、100、200、400、600、800、1 000 μg·mL$^{-1}$) 的溶液加入, 放入细胞培养箱孵育24 h, 然后每孔加入10 μL CCK8试剂, 孵育2 h, 用Infinite M1000Pro多功能酶标仪于450 nm处测吸光度值。

**LDH检测**　给药和造模后, 收集细胞上清液用于LDH的检测。LDH的检测方法按照试剂盒说明书进行, 在90 μL细胞上清液中加入60 μL LDH检测工作液, 放在振荡仪上以300 r·min$^{-1}$的转速室温避光振荡30 min, 在490 nm处用Infinite M1000Pro酶标仪测定吸光度值。

**细胞形态观察**　细胞用不同组别的药物刺激后, 在普通相差显微镜下观察细胞形态, 并随机选择视野进行拍摄。

**DAF-FM DA探针检测NO**　按1∶1 000的比例用试剂盒提供的DAF-FM DA稀释液稀释DAF-FM DA, 使终浓度为5 μmol·L$^{-1}$, 去除细胞培养液, 每孔加入80 μL的5 μmol·L$^{-1}$ DAF-FM DA溶液, 孵育20 min。用PBS洗涤细胞3次, 充分去除未进入细胞内的DAF-FM DA, 在Image Xpress Micro Confocal高内涵成像系统中用FITC通道进行拍摄, 每孔9个视野, 分析细胞的荧光强度。

**DCFH-DA探针检测活性氧**　细胞给药和造模后, 弃去上清, 用PBS洗2遍, 加入20 μmol·L$^{-1}$ DCFH-DA溶液放入细胞培养箱孵育30 min, PBS洗3遍后, 在Image Xpress Pico自动细胞成像系统中用FITC通道进行拍摄, 每个孔9个视野, 分析细胞的荧光强度。

**TMRM染料检测线粒体膜电位**　细胞给药和造模后, 弃去上清, 用PBS洗2遍, 加入20 nmol·L$^{-1}$ TMRM溶液孵育30 min, 然后用Image Xpress Micro Confocal高内涵成像系统采集图像, 每个孔4个视野, 分析细胞的荧光强度。

**单核细胞黏附实验**　内皮细胞给药和造模后, 弃去上清, 加入Hoechst 33342染色液孵育10 min, 充分洗涤后, 加入用5 μmol·L$^{-1}$ Calcein-AM标记过并清洗干净的THP-1细胞孵育1 h, 弃上清洗3遍, 用Image Xpress Micro Confocal高内涵成像系统采集图像, 每个孔4个视野, 并分别计数HUVECs和THP-1细胞。

**ELISA法检测黏附因子、炎症因子和趋化因子**　细胞给药和造模后, 取上清用于ICAM-1、IL-6、MCP-1的检测。ELISA的实验方法按试剂盒的说明书进行。每个实验处理组设置3个复孔, 3次平行实验。

**统计学分析**　所有数据均以均值 ± 标准差 ($\bar{x} \pm s$) 表示, GraphPad Prism 9.0.0软件作图, 两组间采用student's *t*-test分析进行比较, 多组间采用one-way ANOVA分析比较。$P < 0.05$说明差异具有统计学意义。

为了探究丹参和川芎是否具有协同效应, 本研究采用Bliss独立模型[26]计算两个化合物的协同指数, 观察到的药物组合效果表示为$E_{AB}$, A和B的理论加和效应为$E_A+E_B\ (1-E_A) = E_A+E_B-E_AE_B$。协同效应指数(combination index, CI) = $(E_A+E_B-E_AE_B)/E_{AB}$, 当CI < 1时, 联合效应被认为高于理论加和效应。

## 结果

### 1　ox-LDL损伤HUVECs细胞的模型构建

用不同浓度的ox-LDL刺激HUVECs细胞24 h, 可以发现细胞存活率呈现先升高后降低的趋势 (图1A), 在低浓度 (30、50 μg·mL$^{-1}$) 时, ox-LDL促进HUVECs细胞的增殖, 而在高浓度 (80、100 μg·mL$^{-1}$) 时, ox-LDL损伤并降低HUVECs细胞的存活率。从LDH、ICAM-1和IL-6释放量来看 (图1B～D), 当ox-LDL浓度为100 μg·mL$^{-1}$时, 该组数值显著高于对照组, 说明此时细胞膜产生明显破裂导致LDH大量渗出, 并且发生严重的炎性损伤, 故后续实验采用100 μg·mL$^{-1}$ ox-LDL浓度对HUVECs进行造模损伤。

### 2　冠心宁片对HUVECs细胞具有促增殖作用

用不同浓度的冠心宁作用于内皮细胞 (图2), 发现当冠心宁浓度为400和600 μg·mL$^{-1}$时对细胞有显著的促增殖作用 ($P < 0.01$), 而当浓度升高到1 000 μg·mL$^{-1}$时, 活细胞数量又明显下降, 说明此时浓度过高, 不利于内皮细胞的生长。所以在后续的损伤实验中, 冠心宁浓度在400～600 μg·mL$^{-1}$用来探究保护作用较为合适。

### 3　冠心宁片及丹参−川芎对细胞形态具有改善作用

冠心宁片由丹参和川芎两味药材的提取物组成, 为了探究冠心宁片及丹参、川芎提取物各自的保护作用, 在ox-LDL损伤HUVECs细胞模型中, 给药组除了设置400 μg·mL$^{-1}$冠心宁, 还设置了该浓度下对应含量的丹参提取物组 (124.8 μg·mL$^{-1}$) 和川芎提取物组 (185.6 μg·mL$^{-1}$)。从细胞形态上来看 (图3), 对照组细胞形态完整饱满, 细胞状态良好, 而模型组经过ox-LDL的损伤后细胞皱缩, 呈现碎片化, 冠心宁、丹参和川芎组细胞形态较模型组更完整, 说明对损伤有一定的改善。

### 4　丹参−川芎对HUVECs细胞NO水平的提高具有协同增效作用

NO是衡量内皮功能的一个重要指标。当内皮功能障碍发生时, NO的产生明显下降。NO探针DAF-FM DA进入细胞后被细胞内的酯酶催化形成不能穿过细胞膜的DAF-FM。DAF-FM本身仅有很弱的荧光, 但在和NO反应后可以产生强烈荧光。从成像结果来看 (图4A), ox-LDL损伤后细胞的荧光强度明显下降, 说明NO的产生明显减少。从统计结果来看 (图4B、C), 冠心宁和对应浓度的丹参、川芎组均能显著提高NO的产生量 ($P < 0.000\ 1$), 且协同效应指数为0.879 0, 小于1, 说明丹参−川芎的联合使用对改善内皮功能障碍具有协同效应。

### 5　冠心宁片及丹参−川芎显著改善内皮细胞的氧化应激状态

ox-LDL对内皮细胞的损伤还体现在氧化损伤上。用ROS水平来反映细胞的氧化损伤程度, ROS水平越高, DCFH-DA表征下的细胞荧光强度越大。从实验结果来看 (图5), 模型组的ROS水平明显高于对照组, 而冠心宁组、丹参组和川芎组则明显降低, 说明冠心宁及其药效物质丹参、川芎均有显著的改善氧化损伤作用 ($P < 0.05$)。

### 6　冠心宁片及丹参−川芎缓解ox-LDL对内皮细胞的炎性损伤

ox-LDL刺激内皮细胞产生黏附因子, 使单核细胞能黏附在内皮上, 进而可以迁移到内膜, 是动脉粥样硬化初始阶段的一个重要病理环节。从实验结果来看 (图6A～C), 模型组显著增加了炎症因子IL-6、趋化因

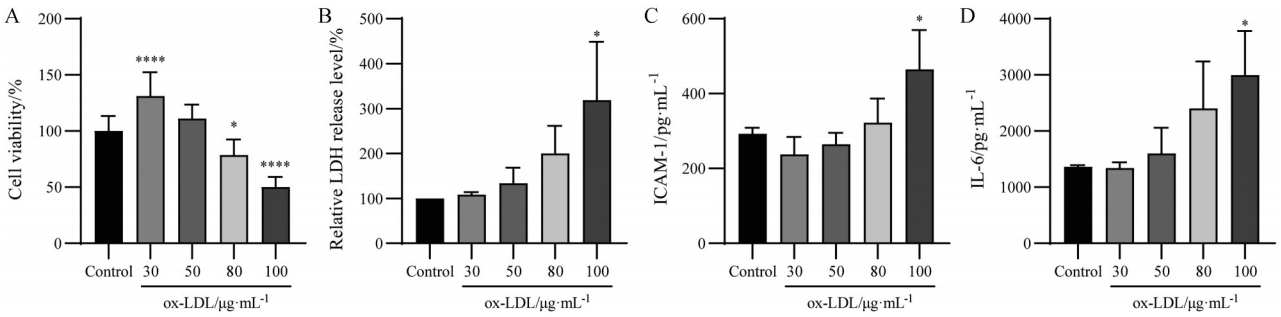


**Figure 1**　Effect of different concentrations of oxidized low density lipoprotein (ox-LDL) in HUVECs. A: HUVECs were respectively treated with ox-LDL (0, 30, 50, 80, 100 μg·mL$^{-1}$) for 24 h, and cell viability was determined by cell counting kit-8 (CCK-8) assay; B: Lactate dehydrogenase (LDH) relative release level was determined by LDH release assay kit; C, D: Intercellular cell adhesion molecule-1 (ICAM-1) and interleukin 6 (IL-6) were detected by ELISA kit. $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{****}P < 0.000\ 1$ *vs* control group

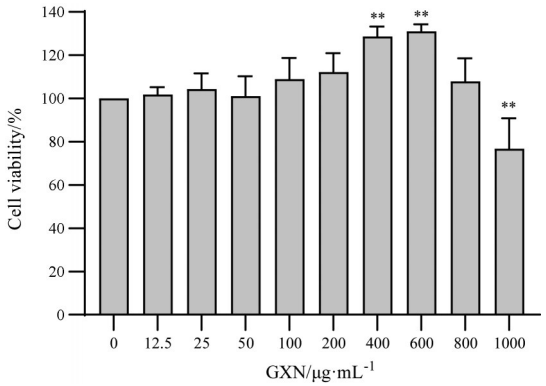


**Figure 2**　Effects of Guanxinning Tablet (GXN) on promoting the proliferation of HUVECs. Cell viability was determined by CCK-8 assay. $n = 3, \bar{x} \pm s$. $^{**}P < 0.01$ *vs* control group

子MCP-1、黏附因子ICAM-1的分泌量 ($P < 0.000\ 1$); 冠心宁、丹参和川芎组对ICAM-1的分泌无显著降低作用, 说明冠心宁对内皮细胞的保护作用不在于抑制ICAM-1; 而冠心宁和对应浓度的丹参组则能显著降低ox-LDL损伤下的MCP-1分泌量, 川芎无显著降低作用, 结合THP-1单核细胞招募实验结果 (图6D、E), 冠心宁和丹参组黏附的THP-1细胞均较模型组显著降低, 说明冠心宁能显著抑制内皮细胞对单核细胞的招募, 在一定程度上减缓动脉粥样硬化的发生, 并且这种作用主要来自于丹参。在炎症因子IL-6水平上 (图6A), 冠心宁及其对应含量的丹参和川芎组均有显著降低作用, 且丹参的降低程度大于川芎。由此可见冠心宁能显著减轻内皮细胞在ox-LDL刺激下的炎症反应, 并且其中的丹参和川芎均发挥作用, 丹参的作用优于川芎。

## 7　丹酚酸B和丹参素在改善内皮功能障碍中发挥主要作用

由前述结果可知, 冠心宁及其对应含量的丹参、川芎提取物能有效改善ox-LDL损伤下的内皮功能障碍。

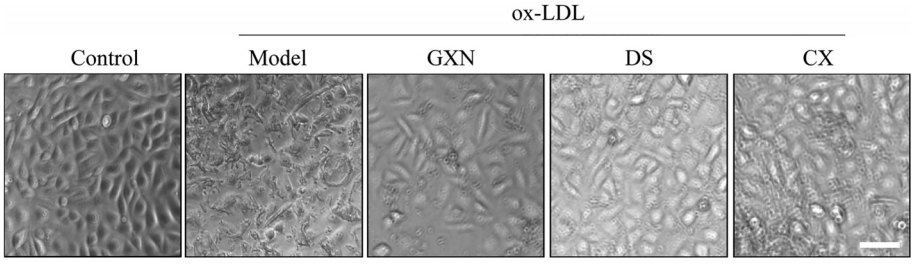


**Figure 3**　GXN and its corresponding contents of *Salvia miltiorrhiza* Bunge (DS) and *Ligusticum striatum* DC. (CX) can improve cell morphology under ox-LDL damage. Exposed to 100 μg·mL$^{-1}$ ox-LDL, HUVECs were also treated with 400 μg·mL$^{-1}$ GXN, 124.8 μg·mL$^{-1}$ DS and 185.6 μg·mL$^{-1}$ CX for 24 h. Scale bar is 50 μm

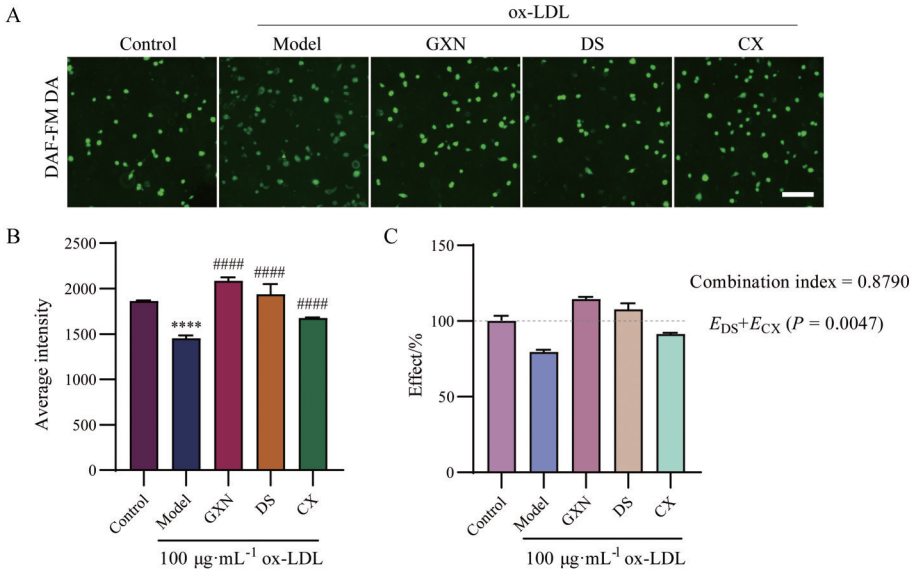


**Figure 4**　GXN and its corresponding contents of DS and CX regulate NO production in HUVECs. A: NO generation was detected with probe DAF-FM DA, scale bar is 100 μm; B: Quantitative results of average intensity of NO fluorescence. $n = 3, \bar{x} \pm s$. $^{****}P < 0.000\ 1$ *vs* control group; $^{\#\#\#\#}P < 0.000\ 1$ *vs* model group; C: The combination index was calculated according to Bliss independence model. CI = $(E_{DS} + E_{CX} - E_{DS} \times E_{CX})/E_{GXN}$. The dotted line represents the value of $E_{DS} + E_{CX} - E_{DS} \times E_{CX}$

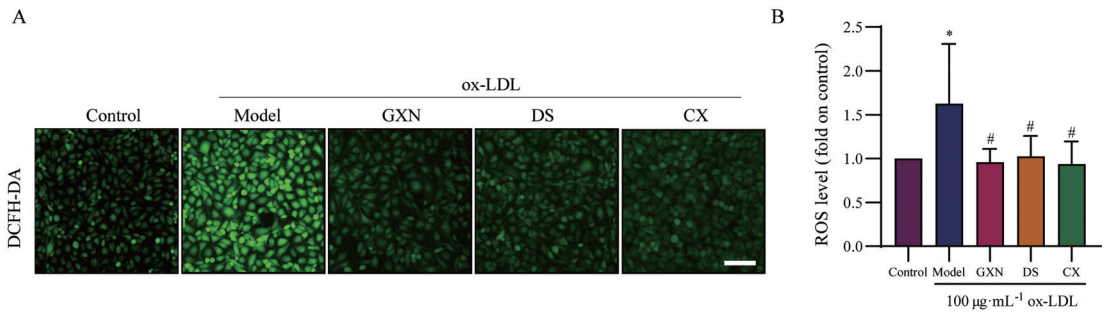


**Figure 5** GXN and its corresponding contents of DS and CX inhibit reactive oxygen species (ROS) production induced by ox-LDL in HUVECs. A: ROS generation in HUVECs was determined by measuring DCFH fluorescence. Scale bar is 100 μm; B: Quantitative results of intensity fold on control group. $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$ *vs* control group; $^{\#}P < 0.05$ *vs* model group

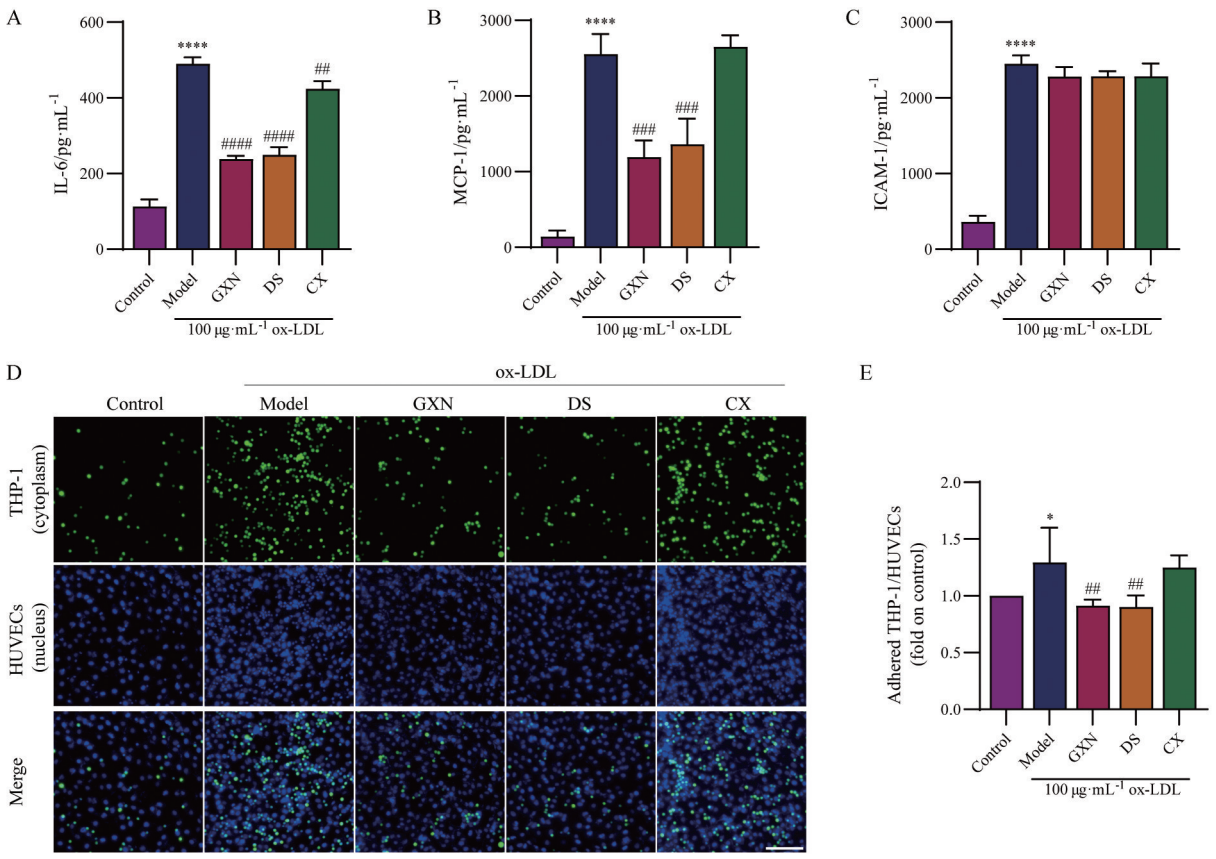


**Figure 6** Effects of GXN and its corresponding contents of DS and CX on THP-1 adhesion and inflammation-related factors secretion in HUVECs treated with ox-LDL. A−C: Levels of interleukin-6 (IL-6, A), monocyte chemoattractant protein-1 (MCP-1, B) and intercellular adhesion molecule-1 (ICAM-1, C) were detected by ELISA assays; D: HUVECs were stimulated by 100 μg·mL$^{-1}$ ox-LDL for 24 h, then stained by Hoechst 33342 to mark nuclei (blue). After washing off the supernatant, HUVECs were co-cultured with fluorescently labeled (green) THP-1 cells for 1 h. Scale bar is 80 μm; E: The adhered THP-1 cells were counted and normalized by HUVECs number. $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{****}P < 0.000\ 1$ *vs* control group; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.000\ 1$ *vs* model group

为了进一步探究起药效的单体化合物, 接下来分别选择了来自丹参的主要化合物丹酚酸B、丹参素、迷迭香酸, 以及来自川芎的主要化合物阿魏酸、洋川芎内酯I进行实验。在NO水平上 (图7A、C), 丹酚酸B和丹参素组较模型组显著提高; 在ROS产生量上 (图7B、D), 丹酚酸B和丹参素组较模型组显著降低。这些结果提示, 丹酚酸B和丹参素在改善ox-LDL引起的内皮功能障碍中发挥主要作用。

## 8 丹参素和阿魏酸联合减轻ox-LDL引发的线粒体损伤

为了进一步探究丹参和川芎的单体成分是否具有联合药效, 丹参中的丹酚酸B、丹参素和迷迭香酸分别

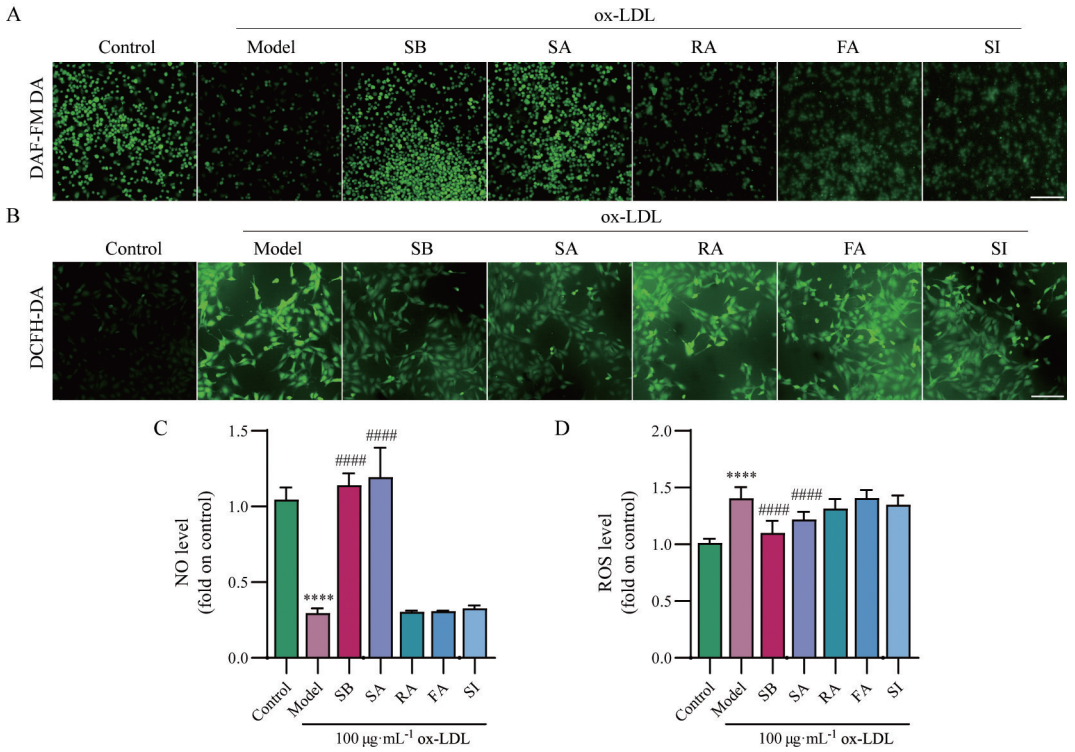


**Figure 7**　Salvianolic acid B (SB) and salvianic acid A (SA) attenuates ox-LDL-induced oxidative stress and endothelial dysfunction. SB, SA and rosmarinic acid (RA) are main compounds derived from DS; ferulic acid (FA) and senkyunolide I (SI) are main compounds derived from CX. Exposed to 100 μg·mL$^{-1}$ ox-LDL, HUVECs were also respectively treated with 10 μmol·L$^{-1}$ SB, SA, RA, FA and SI for 24 h. A: NO generation was detected with probe DAF-FM DA, scale bar is 80 μm; B: ROS generation in HUVECs was determined by measuring DCFH fluorescence, scale bar is 80 μm; C: Quantitative results of (A) intensity fold on control group; D: Quantitative results of (B) intensity fold on control group. $n = 3$, $\bar{x} \pm s$. $^{****}P < 0.000\ 1$ *vs* control group; $^{\#\#\#\#}P < 0.000\ 1$ *vs* model group

与川芎中的阿魏酸、洋川芎内酯I两两组合 (图8A) 进行实验。ox-LDL诱导内皮细胞产生氧化应激, ROS水平升高, 并且伴随着线粒体膜电位的降低, 故可用线粒体膜电位水平反映内皮细胞损伤程度。TMRM是一种亲脂性阳离子染料, 线粒体膜电位越高, 荧光强度越大。从实验结果来看 (图8B), “丹酚酸B”、“丹参素”、“丹酚酸B + 阿魏酸”、“丹参素 + 阿魏酸”和“丹酚酸B + 洋川芎内酯I”这5组均具有提高线粒体膜电位的作用, 其中“丹参素 + 阿魏酸”联合给药组的药效显著高于“丹参素”单给药组 (图8B、C) ($P < 0.05$), 即丹参素和阿魏酸的联合药效优于单给药药效。

综上所述, 实验发现冠心宁片及其对应含量的丹参、川芎提取物均具有改善内皮功能、抵抗氧化应激、缓解炎性损伤的作用, 并且丹参和川芎协同提升内皮基础功能, 而丹参在多个指标上发挥着更强效的作用; 在药效物质研究中, 丹酚酸B和丹参素发挥主要的抗氧化和改善内皮功能障碍作用, 并且丹参素和阿魏酸的联合药效优于单给药药效。上述结果提示冠心宁片可能通过调控氧化应激及炎性损伤改善内皮功能障碍, 缓解动脉粥样硬化的形成。

## 讨论

中药防治AS具有独特优势, 能够从多途径、多环节、多靶点干预AS的发生发展[27,28]。其中一种保护机制是通过抑制炎症因子的产生来抵抗AS。有研究报道长期摄入杜仲叶提取物可以通过提高血浆NO水平, 并抑制ICAM-1和VCAM-1的产生来改善血管功能[29]。同时, Hosoo等[30]发现杜仲提取物能显著改善自发性高血压大鼠的主动脉内皮依赖性松弛, 在给药组中, 血浆NO水平显著增加, 表明杜仲叶提取物可能发挥抵抗内皮功能障碍和AS的作用。Yang等[31]研究了何首乌水溶性成分何首乌二苯乙烯苷 (*Polygonum multiflorum* stilbene glycoside, PMS) 对巨噬细胞衍生的泡沫细胞的作用, 发现PMS可以降低氧化脂蛋白诱导的ICAM-1和血管内皮生长因子 (vascular endothelial growth factor, VEGF), 表明PMS是一种强大的抗AS药物, 并且PMS的作用可能是通过抑制泡沫细胞中ICAM-1和VEGF的产生。MCP-1及其受体在招募单核细胞到内皮进而形成泡沫细胞的过程中起着关键作用。Guo等[32]发现川芎中的活性成分川芎嗪 (tetramethyl-

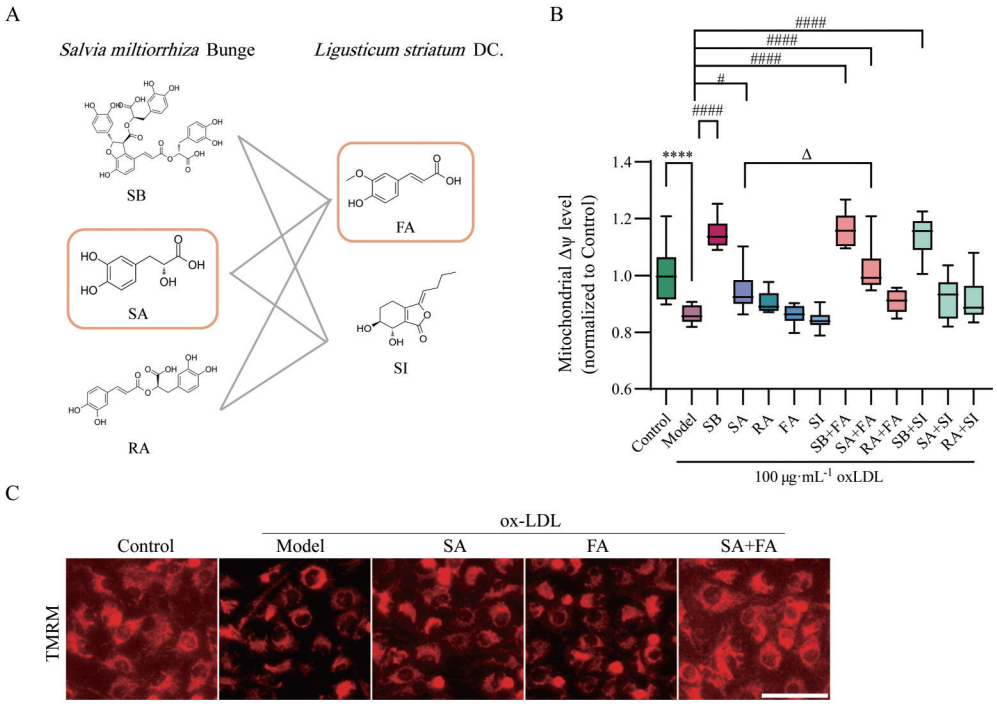


**Figure 8**　Combined efficacy of compounds from DS and CX. A: Molecular structure of main compounds derived from the two herbs. Grey lines illustrated the compound combinations tested and yellow box outlined the two compounds with positive interaction detected in our assay; B: Quantitative results showing the mitochondrial protective effects of main compounds and compound combinations. The dosages were all 10 μmol·L$^{-1}$, for both single compound and compound combination treatments; C: Images of mitochondrial membrane potential detected by probe TMRM. The interaction between SA and FA was examined. Scale bar is 40 μm. $n$ = 10, $\bar{x} \pm s$. $^{****}P < 0.000\ 1$ *vs* control group; $^{\#}P < 0.05$, $^{\#\#\#\#}P < 0.000\ 1$ *vs* model group; $^{\Delta}P < 0.05$ *vs* SA group

pyrazine, TMP) 可降低兔血浆中的MCP-1水平并抑制兔主动脉中LOX-1的产生。同样, 他们在体外的研究中发现TMP抑制ox-LDL诱导的p-ERK、p-p38和p-JNK的活化, 证明TMP保护内皮并通过抑制免疫反应发挥抗AS作用[33]。

另一种广泛报道的中草药抗AS机制是通过Nrf2/HO-1信号通路的激活来保护内皮细胞免受氧化应激损伤。Xiong等[34]利用双氧水诱导HUVECs细胞模型, 发现临床常用治疗心血管疾病的中药方剂血栓心脉宁片 (XXT) 能够降低HUVECs中的ROS水平, 其潜在机制与Nrf2和下游蛋白 (HMOX、GCLM、NQO1) 的调节有关。黄烷醇是自然界植物中广泛分布的一种具有多生物活性的黄酮类化合物。二氢杨梅素 (dihydromyricetin, DMY) 是提取自葡萄科蛇葡萄属的天然黄烷酮。研究发现, DMY可激活Akt和ERK1/2, 诱导Nrf2/HO-1信号传导, 恢复线粒体膜电位, 抑制内皮细胞凋亡, 保护HUVECs免受ox-LDL诱导的氧化损伤[35]。除此之外, DWY可以逆转HUVECs细胞焦亡并抑制caspase-1活化、IL-1$\beta$释放和NLRP3炎性小体活化, 还通过激活Nrf2信号通路在血管内皮细胞焦亡中发挥重要作用[36]。

在本研究中, 高浓度的ox-LDL诱导HUVECs分泌更多的炎症因子, 致使细胞膜破裂释放出更多的LDH, 细胞活力下降, 而冠心宁能保护内皮细胞的完整性, 促进内皮细胞增殖, 在增加内皮细胞产生NO的同时, 还能显著抑制ROS水平, 减少炎症因子IL-6和趋化因子MCP-1的分泌。把组成冠心宁的丹参和川芎提取物分别进行实验, 发现两味药材都能发挥抗氧化应激和抗炎的作用, 但是有效程度并不相同, 在多个指标上丹参更加强效。在内皮细胞的基础功能上, 丹参和川芎协同增效。为了进一步探究冠心宁片中具体哪些成分起效, 本研究选择了丹酚酸B、丹参素、迷迭香酸、阿魏酸和洋川芎内酯I 5种主要化合物分别进行单给药实验和联合给药实验, 在单给药实验中, 丹酚酸B和丹参素发挥抗氧化和改善内皮功能障碍的主要作用; 在联合给药实验中, 丹参素和阿魏酸的联合药效优于单给药药效。

综合上述, 本研究的结果提示冠心宁片及其组成的丹参、川芎提取物具有改善内皮基础功能、抵抗氧化应激、缓解炎性损伤的作用, 并且丹参和川芎协同增效, 其中的药效物质丹酚酸B和丹参素发挥主要作用, 并且丹参素和阿魏酸的联合药效优于单给药药效。本研

究为冠心宁片的抗动脉粥样硬化应用提供实验依据。

**作者贡献**: 王毅、赵璐和王木兰负责提出研究选题、设计研究方案及文章的修改; 余敏负责研究实验的开展和论文的撰写。

**利益冲突**: 全体作者声明不存在任何利益冲突。

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