论著·基础医学

麝香酮通过促进VEGF表达介导的微血管生成改善冠状动脉微血管疾病

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[摘要] **目的** 探讨麝香保心丸(shexiang baoxin pill, SXBXP)对冠状动脉微血管疾病(coronary microvascular disease,CMD)的影响以及麝香酮(Muscone)作为SXBXP中的有效成分对CMD的作用及机制。**方法** 8周龄的ob/ob小鼠按照每天10 mg/kg SXBXP(SXBXP组)或无菌PBS(Ctl组)进行灌胃,同背景野生型小鼠(WT组)作为阴性对照;以缺血缺氧干预小鼠冠脉微血管内皮细胞(mouse coronary microvascular endothelial cell,MCMEC)构建CMD细胞模型(HI组),并以20 μg/mL浓度的SXBXP(HI+SX)和30 μmol/L浓度的Muscone(HI+Muscone)分别进行药物干预。通过心脏超声动态评估冠状动脉血流储备(coronary flow reserve,CFR)水平和左心室功能,激光散斑成像评估心肌血流量(myocardial blood flow,MBF)水平。通过CD31免疫荧光染色评估小鼠心脏微血管密度。通过免疫荧光染色和Western blot检测心脏微血管内皮细胞的血管内皮生长因子(Vascular endothelial growth factor,VEGF)表达。5-乙炔基-2-脱氧嘧啶核苷染色、血管生成实验检测MCMEC的增殖能力和成管能力。**结果** ob/ob-Control组小鼠CFR、MBF水平和心脏微血管密度均比WT组小鼠显著降低($P<0.01$),ob/ob-SXBXP组小鼠的上述指标均显著高于ob/ob对照组($P<0.05$),表明SXBXP对CMD表型具有改善作用。ob/ob-Control组小鼠心脏微血管内皮细胞VEGF表达水平比WT组小鼠显著降低($P<0.05$),而ob/ob-SXBXP组小鼠心脏微血管内皮细胞VEGF表达水平显著高于ob/ob-Control组($P<0.05$),SXBXP可能通过VEGF介导的微血管生成改善CMD。体外实验进一步显示,缺血缺氧组(HI)组的VEGF表达水平及MCMEC的增殖和成管能力均显著低于常氧培养(Ctl组)($P<0.05$),缺血缺氧SXBXP干预组(HI+SX)和缺血缺氧Muscone干预组(HI+Muscone)上述指标均显著高于HI组($P<0.05$),Muscone作为SXBXP的活性物质通过VEGF介导的微血管生成改善CMD。**结论** SXBXP对CMD具有改善作用;Muscone作为SXBXP中的主要成分通过诱导VEGF的表达促进冠状动脉微血管生成,增加心肌灌注水平从而改善CMD。

[关键词] 冠状动脉微血管疾病;麝香酮;麝香保心丸;微血管生成;血管内皮生长因子。
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Muscone improves coronary microvascular disease by promoting VEGF-mediated microvascular angiogenesis

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[**Abstract**] Objective To explore the impact of Shexiang Baoxin Pill (SXBXP, a traditional Chinese patent medicine, broadly applied for the treatment of cardiovascular diseases) on coronary microvascular disease (CMD) and investigate the role and underlying mechanisms of muscone, a key bioactive component of the pills, in the treatment. Methods A total of 16 ob/ob mice (8 weeks old) were randomly and equally divided into an ob/ob-SXBXP group and a ob/ob-Control group, receiving 10 mg/kg·d SXBXP or PBS *via* oral gavage, and another 8 wild-type mice with the same genetic background (WT group) were subjected as a

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negative control group. Cell model of CMD was established based on mouse coronary microvascular endothelial cells (MCMEC) under ischemia and hypoxic (HI) condition, and the cells were further treated with 20 μg/mL SXBXP (HI+SX) and 30 μmol/L muscone (HI+Muscone), respectively. Echocardiography was carried out for coronary flow reserve (CFR) and left ventricular function, and laser speckle imaging was applied to evaluate myocardial blood flow (MBF). Microvascular density in the heart was evaluated with CD31 immunofluorescence staining. The expression levels of vascular endothelial growth factor (VEGF) in cardiac microvascular endothelial cells of both mouse and cell models were detected by immunofluorescence staining and Western blot analysis. The proliferation and angiogenesis of MCMEC were observed by 5-ethynyl-2′-deoxyuridine staining and angiogenesis experiments. **Results** In the ob/ob-Control group of mice, the levels of CFR, MBF, and cardiac microvascular density were significantly lower than those in the WT group ($P<0.05$), and these indicators were significantly improved in the ob/ob-SXBXP group when compared with the ob/ob-Control group ($P<0.05$), which indicates that SXBXP improves the CMD phenotype. The expression level of VEGF in cardiac microvascular endothelial cells was significantly lower in the ob/ob-Control mice than the WT mice ($P<0.05$), while the level in the ob/ob-SXBXP group was significantly higher than that in the ob/ob-Control group ($P<0.05$), illustrating that SXBXP may ameliorate CMD through VEGF-mediated microvascular angiogenesis. *In vitro* experiments further revealed that the VEGF expression level and the proliferation and angiogenesis abilities in MCMEC were significantly lower in the HI group than the cells under the normoxia control condition ($P<0.05$). Both SXBXP and muscone treatment resulted in enhanced expression of VEGF and improved proliferative and angiogenesis abilities of MCMEC ($P<0.05$). These results suggest that muscone could improve CMD by VEGF-mediated microvascular angiogenesis. **Conclusion** SXBXP can improve CMD. Muscone, as a key component of SXBXP, promotes microvascular angiogenesis by inducing the expression of VEGF, then enhances myocardial perfusion, and consequently alleviates CMD.

[**Key words**] coronary microvascular disease; Shexiang Baoxin Pill; muscone; microvascular angiogenesis; vascular endothelial growth factor

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冠状动脉微血管疾病(coronary microvascular disease, CMD)是指发生于前毛细血管和毛细血管水平的结构与/或功能异常,导致冠状动脉血流受损和心肌缺血[1]。CMD是影响冠状动脉疾病(coronary artery disease, CAD)患者预后的独立危险因素[2-4],尽管近年来CAD的诊断和治疗取得了长足进步,但CAD的死亡率仍持续上升[5],其中一个潜在的重要原因是长期以来对CMD诊断和治疗的忽视。50%~70%的CAD患者同时患有CMD,绝经后女性的患病率尤其高[6-7]。目前,CMD的发病机制仍不清楚,且缺乏针对性的干预措施[1,8]。因此,探索CMD的发病机制并寻找针对性的干预措施对于改善CAD患者的预后具有重要意义。

麝香保心丸(shexiang baoxin pill,SXBXP)是一种源自《太平惠民和剂局方》中的中药复方制剂[9-10],包含人工麝香、人参、人工牛黄、肉桂、安息香、蟾酥、冰片等7种成分[11-12],对缓解稳定型心绞痛和心肌缺血引起的胸痛具有多效性作用,包括减轻动脉粥样硬化病变中的炎症[13]、抑制氧化应激[14]以及对抗心脏重构[12]。有研究表明SXBXP具有促进心脏血管生成进而增强心肌灌注的潜力[15-16],并发现SXBXP主要是通过增加内皮细胞对NO合酶的表达,抑制其炎症反应,改善微血管内皮细胞功能,促进微血管生成[17]。麝香作为SXBXP中最重要的物质具有抗氧化、抗炎、血管扩张和血管生成作用[18-19],麝香的主要生物活性成分是麝香酮(Muscone),含量高达2%~5%,被认为是其中最主要的药理活性和气味成分[20],具有心脑血管保护活性、神经保护活性等多种生物学影响[21]。但在CMD的治疗中Muscone作为SXBXP中的主要活性成分缺乏相关机制的探讨。本研究通过构建ob/ob-CMD小鼠模型,明确SXBXP干预对CMD的改善作用,并联合动物模型及小鼠冠状动脉微血管内皮细胞(mouse coronary microvascular endothelial cell, MCMEC)探讨Muscone作为SXBXP中的主要活性物质改善CMD的可能机制,为后续进一步挖掘针对性的CMD干预手段提供理论基础和实验依据。

1 材料与方法

1.1 实验药品

SXBXP由上海和黄药业有限公司(生产批号:170716;药品批准文号Z31020068)提供,其质量控制符合《中华人民共和国药典》(中国药典委员会,2015年)中所述的规格和检测程序。根据SXBXP临床剂量推荐

(67.5~135 mg/d),根据体表面积换算法(系数0.026)计算小鼠等效剂量。将SXBXP溶解在生理盐水中,每天取10 mg/kg,用于小鼠灌胃。

1.2 实验动物

雄性C57BL/6 J-lepob小鼠(即ob/ob小鼠,体质量18~22 g,8周龄)和C57BL/6J野生型小鼠(体质量38~42 g,8周龄)均购自南京君科生物科技有限公司。所有小鼠饲养于陆军军医大学高原医学系SPF级动物房,环境温度控制在(22±2)℃,相对湿度为(50±10)%,光照周期为12 h光照/12 h黑暗,按自由摄食标准予啮齿类动物饲料并自由饮水。将8只8周龄的C57BL/6J野生型小鼠作为阴性对照组(WT);16只8周龄ob/ob小鼠随机分为2组:SXBXP干预组(ob/ob-SXBXP)和CMD对照组(ob/ob-Control),每天分别给予SXBXP溶液(10 mg/kg)和PBS灌胃,持续3个月。本研究经陆军军医大学动物伦理委员会的批准(AMUWEC20232970)。同时实验部分严格遵循国家实验动物管理保护条例,实验数据不违反伦理学条例要求。实验全程采用以下措施减少动物痛苦:所有侵入性操作均在超净台内进行严格无菌操作;取材前10 min通过腹腔注射1%戊巴比妥钠进行麻醉;每周进行2次体质量监测评估动物健康状况。

1.3 细胞培养

MCMEC(PAMC-C132,海星生物,中国)在DMEM完全培养基(ECM,Sciencell,中国)中培养,培养基中添加了10%的胎牛血清(A5669701,Gibco,美国)、1%的青霉素/链霉素溶液(BL505A,Biosharp,中国),细胞密度达到80%时给予不同干预。将SXBXP置于无菌PBS中,并用超声破碎仪处理,配制成为2 mg/μL的SXBXP溶液(以20 μg/μL为培养基中药物处理的终浓度[22-23]),将Muscone(541-91-3,道斯夫,中国)溶解在二甲亚砜(67-68-5,Biosharp,中国)中配制成为30 mmol/L的Muscone溶液,并经细菌过滤器抽滤后储存在-20 ℃中备用。在六孔细胞培养板内接种MCMEC(有或无细胞爬片),分别给予不同处理:以DMEM完全培养基在常氧培养箱中培养为阴性对照组(Ctl)、以缺血缺氧(无血清培养基、1%缺氧处理48 h)培养为CMD细胞模型(HI)、以缺血缺氧培养并施加SXBXP(20 μg/mL)为干预组(HI+SX)、以缺血缺氧培养并施加Muscone(参考文献中用药浓度确定Muscone浓度为3 μmol/L[24-25])为Muscone干预组(HI+Muscone)。

1.4 超声心动图

使用VINNO V6实时超声仪(OSEI BIO,中国)检测3组小鼠冠脉血流储备(coronary flow reserve,CFR)以评估冠状动脉微循环状态:将小鼠置于气麻机(F500,瑞沃德,中国)麻醉仓中以3%异氟烷进行麻醉诱导,然后仰卧放置在加热板上,并去除胸部和腹部的毛发。通过吸入2.5%异氟烷测量应激期冠脉血流流速,吸入1%异氟烷测量静息期冠状动脉血流流速,以应激期冠脉血流流速除以静息期冠脉血流流速的值记做CFR[26-27];检测左心室射血分数(left ventricular ejection fraction,LVEF)和短轴缩短分数(fractional shortening,FS)以评估左心室功能。

1.5 激光散斑成像

将小鼠用1%戊巴比妥钠麻醉,然后快速进行气管插管,连接小动物呼吸机(F500,瑞沃德,中国),随后继续左侧开胸术完全暴露心脏。使用激光散斑对比成像仪(RFLSI Ⅲ,瑞沃德,中国)对每只小鼠心脏至少3个不同微循环区域的心肌血流量(myocardial blood flow,MBF)进行检测并统计。

1.6 小鼠心脏取材

对心脏进行采样并称重,沿乳头肌平面切开,心尖迅速在液氮中冷冻,后转移至-80 ℃下保存,以备生化分析。其余心脏组织在4 ℃下用4%多聚甲醛(BL539A,Biosharp,中国)固定24 h,然后依次用75%、80%、85%、90%、95%和100%的酒精梯度脱水,并用二甲苯透明化后通过石蜡包埋制成石蜡组织块。

1.7 心脏与细胞的免疫荧光染色

小鼠心脏组织石蜡包块以5 μm厚度进行切片,经过二甲苯脱蜡、梯度水化和柠檬酸钠抗原修复液(BL619A,Biosharp,中国)修复,山羊血清(HY-NP0197,碧云天,中国)37 ℃封闭30 min后,用CD31抗体(AF3628,R&D,美国)工作液孵育4 ℃过夜,然后以驴抗山羊594偶联二抗(Ab150132,Abcam,英国)工作液37 ℃孵育1 h,以DAPI(C1002,碧云天,中国)染细胞核后用抗荧光淬灭封片剂(P0126,碧云天,中国)封片。通过免疫荧光染色对小鼠心脏组织切片和MCMEC细胞爬片检测VEGF表达:使用血管内皮生长因子(Vascular endothelial growth factor,VEGF)抗体(A18675,Abclonal,中国)和CD31抗体(AF3628,R&D,美国)孵育一抗,以驴抗山羊594偶联二抗(Ab150132,Abcam,英国)和山羊抗兔488偶联二抗(A11008,Invitrogen,美国)工作液孵育二抗。

1.8 Western blot检测VEGF表达

将心脏组织用含蛋白酶和磷酸酶抑制剂(HY-K0021,罗氏,美国)的RIPA缓冲液提取蛋白质,通过BCA(P0010,碧云天,中国)检测蛋白质浓度,以5xSDS上样缓冲液(LT101S,雅酶,中国)制备样本。样品在SDS-PAGE凝胶(4%~20%丙烯酰胺,金斯瑞,美国)上分离,然后转移到PVDF膜(10600023,Cytiva,美国)。印迹膜用以下一抗进行孵育:β-actin抗体(PTM-5028,PTM生物,中国)、VEGF抗体(A18675,Abclonal,中国)工作液孵育4 ℃过夜,与辣根过氧化物酶(horseradish Peroxidase,HRP)偶联的二抗(HA1001,华美生物,中国)工作液孵育,然后用化学发光成像系统(ChenidocTouch^{+}Turbo^{+},Biorad,美国)扫描。蛋白质表

达水平以β-肌动蛋白为参照进行归一化分析。

1.9　统计学分析

使用数据以$\bar{x}\pm s$表示SPSS 20.0软件(美国)完成。对所有数据进行了正态性和方差齐性检验。多组间比较采用单因素方差分析。$P<0.05$被认为具有统计学意义。所有图表均使用GraphPad Prism 8.0软件绘制。

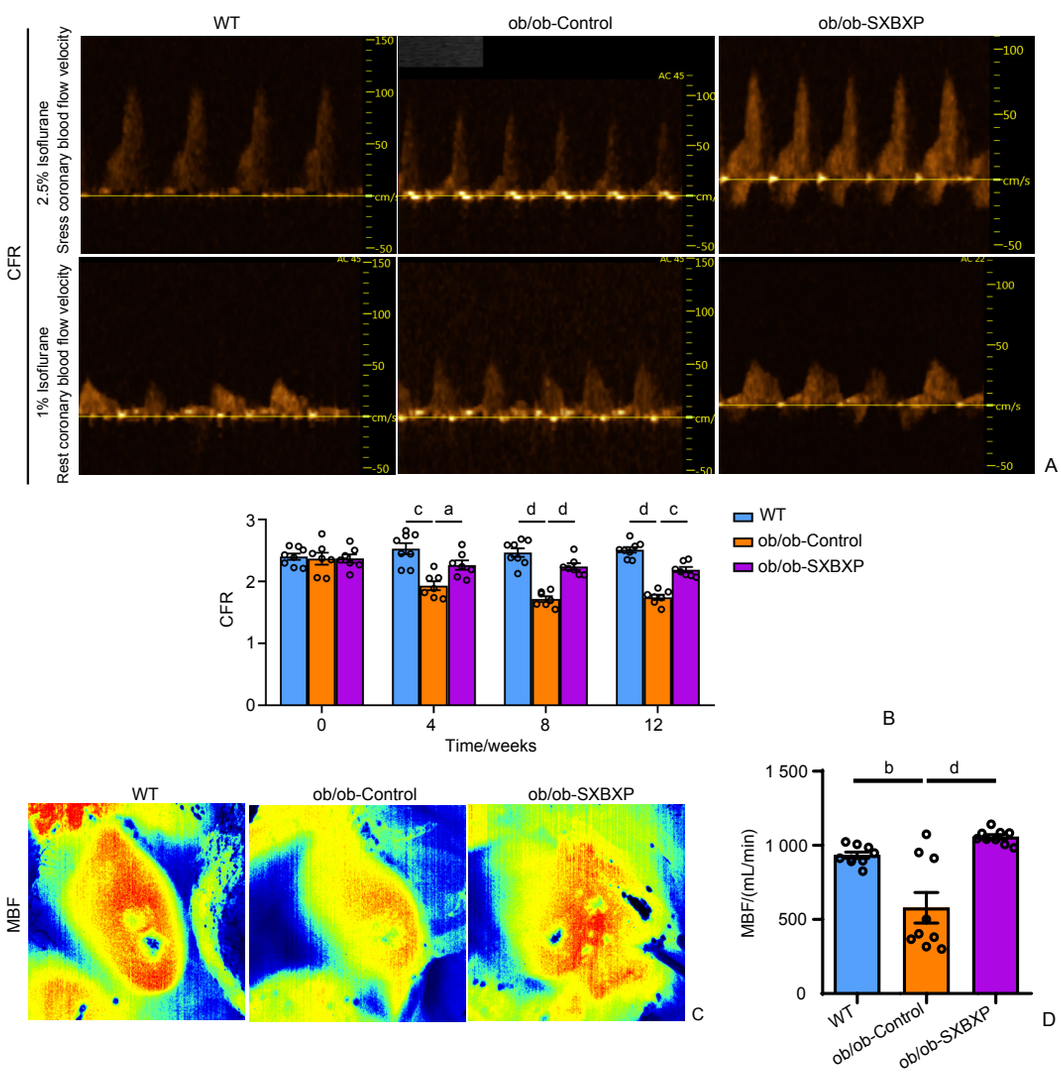
2　结果

2.1　SXBXP干预改善ob/ob-CMD小鼠的CFR和MBF

在第4、8及12周时,ob/ob-Control组小鼠的CFR水平低于WT组,ob/ob-SXBXP组的CFR水平显著高于ob/ob-Control组(图1A、B)。第12周时,ob/ob-Control组的MBF显著低于WT组($P<0.01$),ob/ob-SXBXP组的MBF显著高于ob/ob-Control组($P<0.0001$,图1C、D)。各时间点3组小鼠的EF和FS水平无显著差异(图1E、F)。

2.2　SXBXP改善ob/ob-CMD小鼠的心脏微血管密度

CD31是心脏血管内皮细胞和微血管系统的标志物,统计CD31荧光定量除以细胞核数量代表心脏微血管密度相对水平。ob/ob-Control组小鼠的心脏微血管密度显著低于WT组($P<0.01$),ob/ob-SXBXP组小鼠的心脏微血管密度显著高于ob/ob-Control组($P<0.05$,图2A、B)。相关性分析显示:微血管密度与CFR和MBF正相关(分别为$P<0.05$和$P<0.01$),MBF与CFR也呈正相关($P<0.0001$,图2C)。

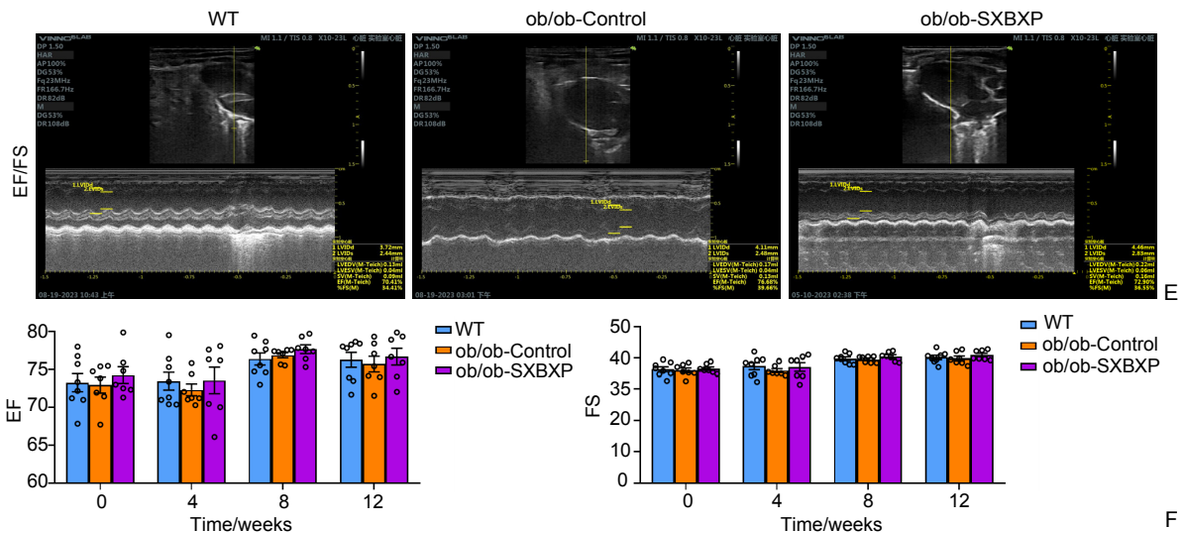


A: Ultrasonic assessment of CFR in mice; B: Bar graph depicting CFR in mice; C: Laser speckle imaging for MBF; D: Bar graph showing MBF in mice; n=8. CFR: Coronary flow reserve; MBF: Myocardial blood flow; EF: Ejection fraction; FS: Fractional shortening. a: $P<0.05$, b: $P<0.01$, c: $P<0.001$, d: $P<0.0001$ compared with the ob/ob-Control group

图1　SXBXP对小鼠CFR、MBF和左心功能的影响

Figure 1　Effects of SXBXP on CFR, MBF, and left heart function in mice

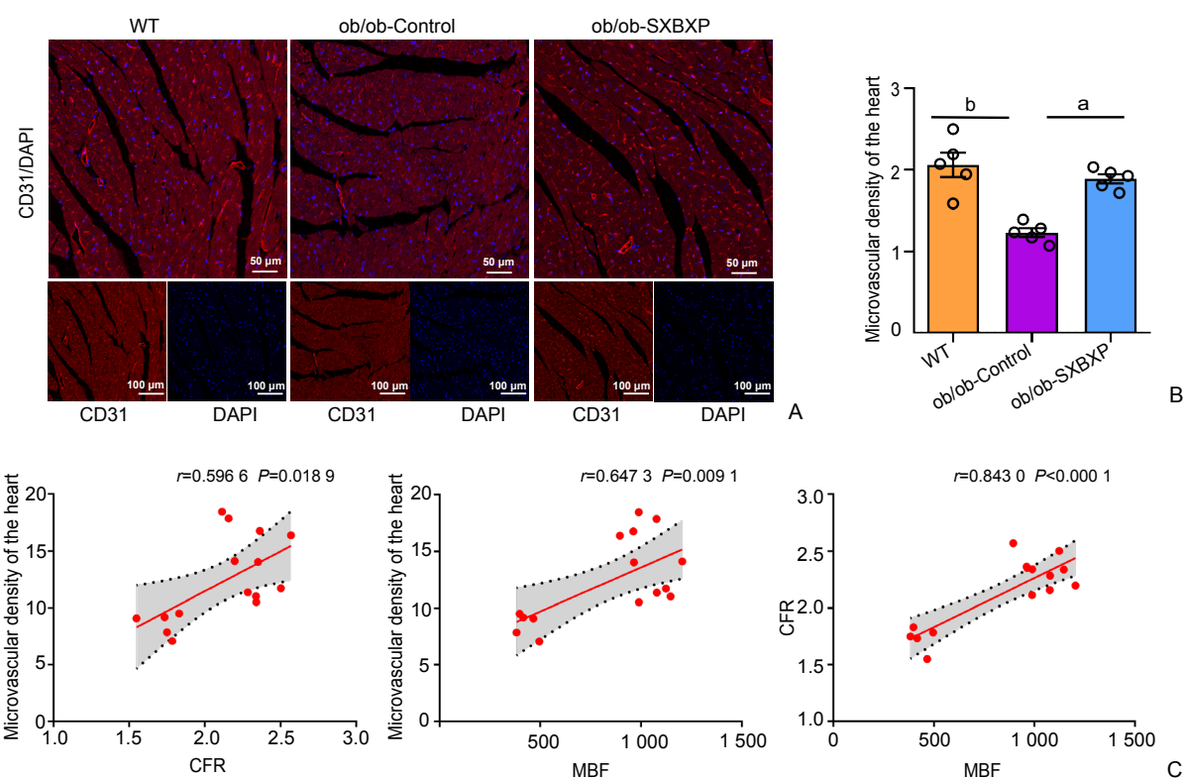
(图1E、F续下页)



E: Echocardiogram for cardiac function in mice; F: Bar graph presenting EF and FS of the hearts in mice (n=8). CFR: Coronary flow reserve; MBF: Myocardial blood flow; EF: Ejection fraction; FS: Fractional shortening. a: P<0.05, b: P<0.01, c: P<0.001, d: P<0.0001, compared with the ob/ob-Control group

续图1 SXBXP对小鼠CFR、MBF和左心功能的影响

Figure 1(continued) Effects of SXBXP on CFR, MBF, heart ventricular function in mice



A: Immunofluorescence staining of microvascular density in the hearts of mice; B: Bar graph depicting the microvascular density in the hearts of mice; C: Correlation analysis of cardiac microvascular density, MBF, and CFR. CFR: Coronary flow reserve; MBF: Myocardial blood flow. a: P<0.05, b: P<0.01, compared with the ob/ob-Control group

图2 小鼠的心脏微血管密度及其与CFR、MBF间的相关性评估

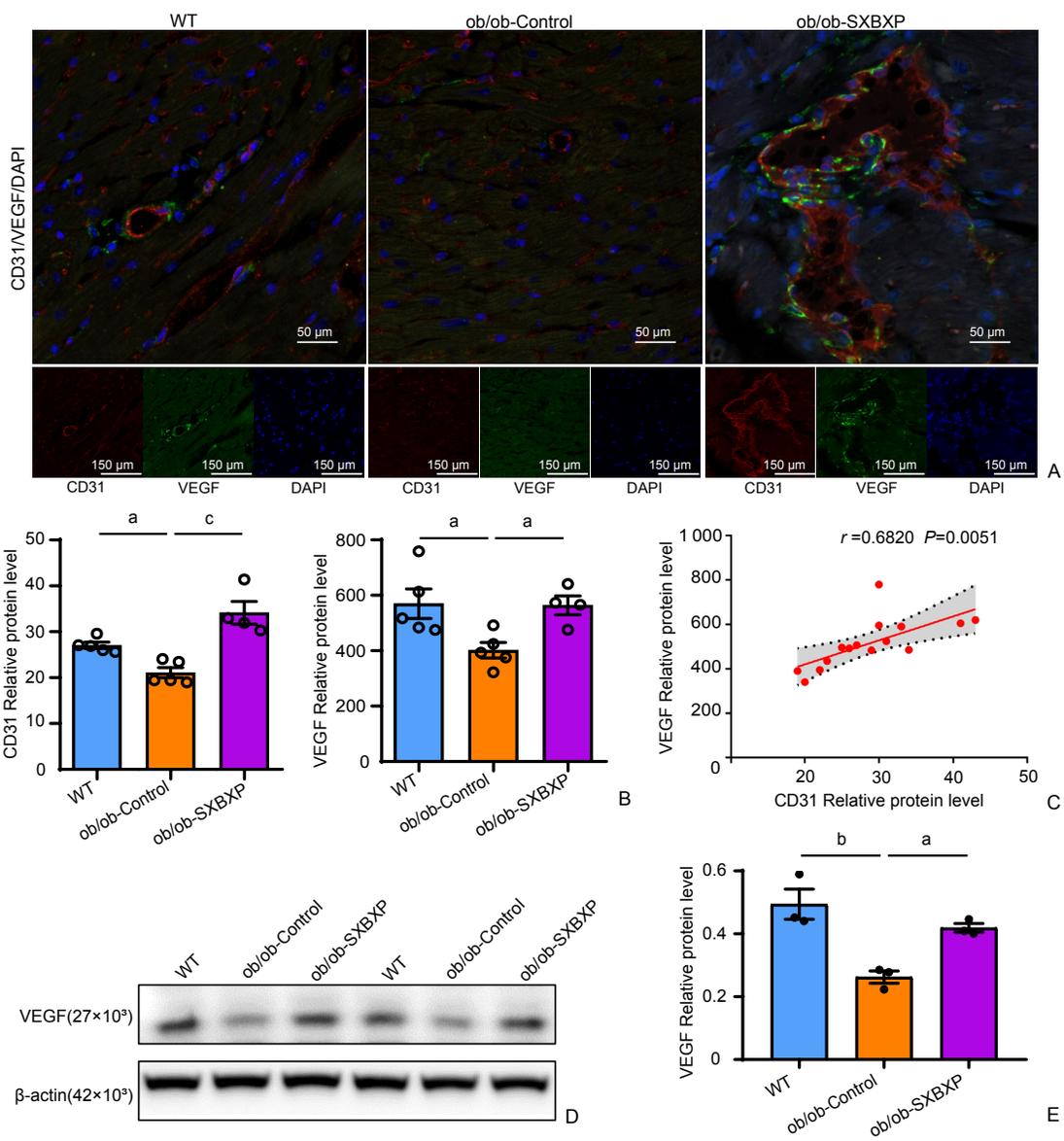
Figure 2 Correlation of cardiac microvascular density with CFR and MBF in mice

2.3　SXBXP 可促进 ob/ob-CMD 小鼠心脏微血管内皮细胞 VEGF 表达上调

CD31 与 VEGF 共染结果显示,ob/ob-Control 组心脏血管内皮细胞中 VEGF 表达水平显著低于 WT 组,ob/ob-SXBXP 组 VEGF 的表达水平显著高于 ob/ob-Control 组(P<0.05,图 3A、B)。相关性分析显示,CD31 表达水平和 VEGF 表达水平显著正相关(P<0.01,图 3C)。此外,心脏组织中 VEGF 蛋白水平检测结果显示:ob/ob-Control 组心脏组织中 VEGF 表达水平显著低于 WT 组(P<0.01),ob/ob-SXBXP 组则显著高于 ob/ob-Control 组(P<0.05,图 3 D、E)。

2.4　SXBXP 增加 MCMEC 的 VEGF 表达,提高细胞增殖能力和成管能力

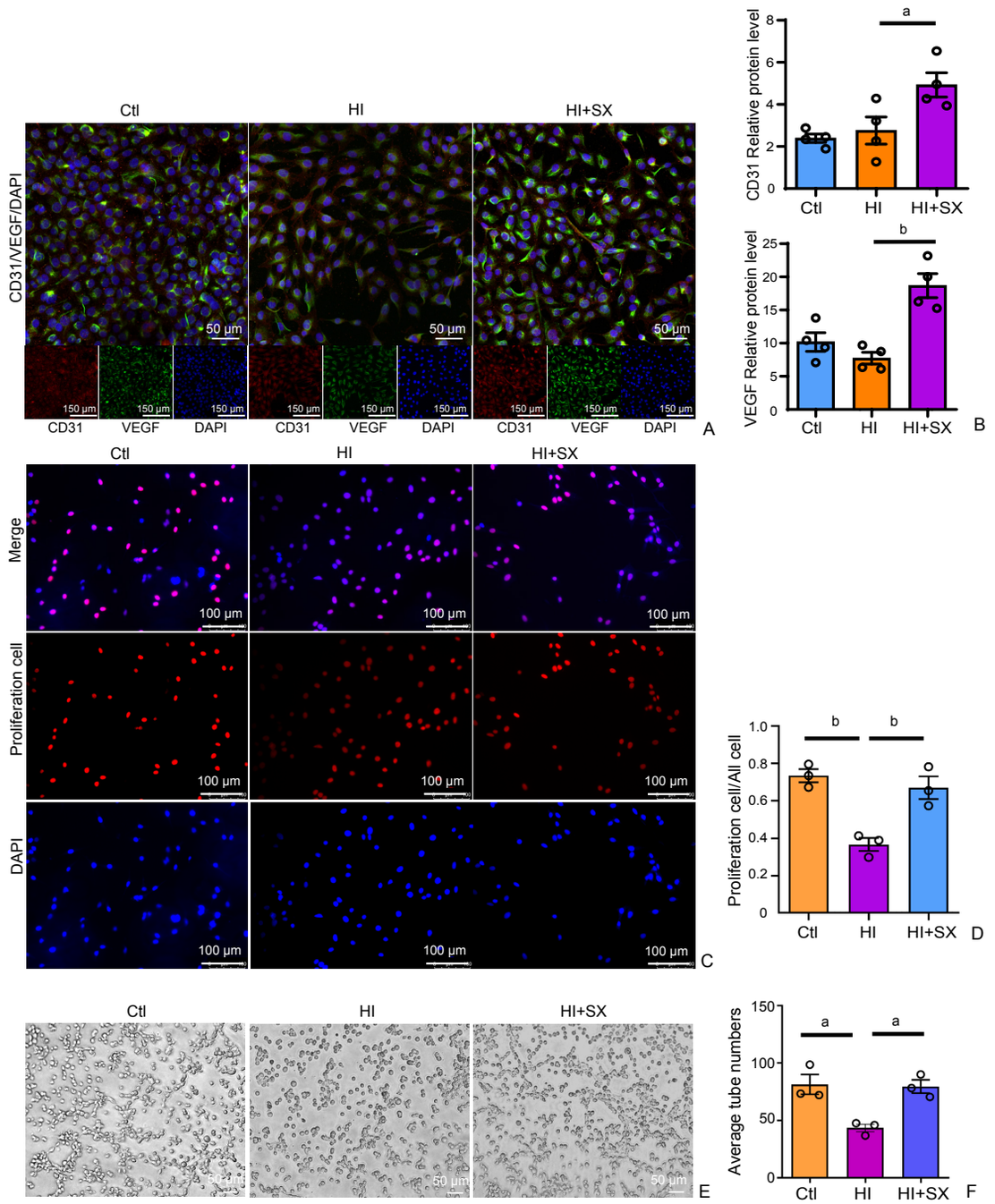
检测 3 组 MCMEC 中 VEGF 的表达水平,结果显示 HI+SX 组中 VEGF 的表达显著高于 HI 组(P<0.05,图 4A、B)。增殖实验发现 HI 组的细胞增殖能力显著低于 Ctl 组(P<0.01),而 HI+SX 组的增殖能力显著高于 HI 组(P<0.01,图 4 C、D)。成管实验发现 HI 组的微血管生成能力显著低于 Ctl 组(P<0.05),而 HI+SX 组的成管能力显著高于 HI 组(P<0.05,图 4E、F)。



A: Double fluorescence staining for CD31 and VEGF in the hearts; B: Quantitative results of double fluorescence staining of CD31 and VEGF in the hearts; C: Correlation analysis between the relative protein levels of CD31 and VEGF in the mice heart; D: Western blotting for VEGF expression in the hearts; E: Relative expression of VEGF protein in the hearts. SXBXP: Shexiang Baoxin Pills; VEGF: Vascular endothelial growth factor. a: P<0.05, b: P<0.01 compared with the ob/ob-Control group

图 3　SXBXP 干预对小鼠心肌组织中 VEGF 表达水平的影响

Figure 3　Effect of SXBXP intervention on the expression level of VEGF in the heart of mice



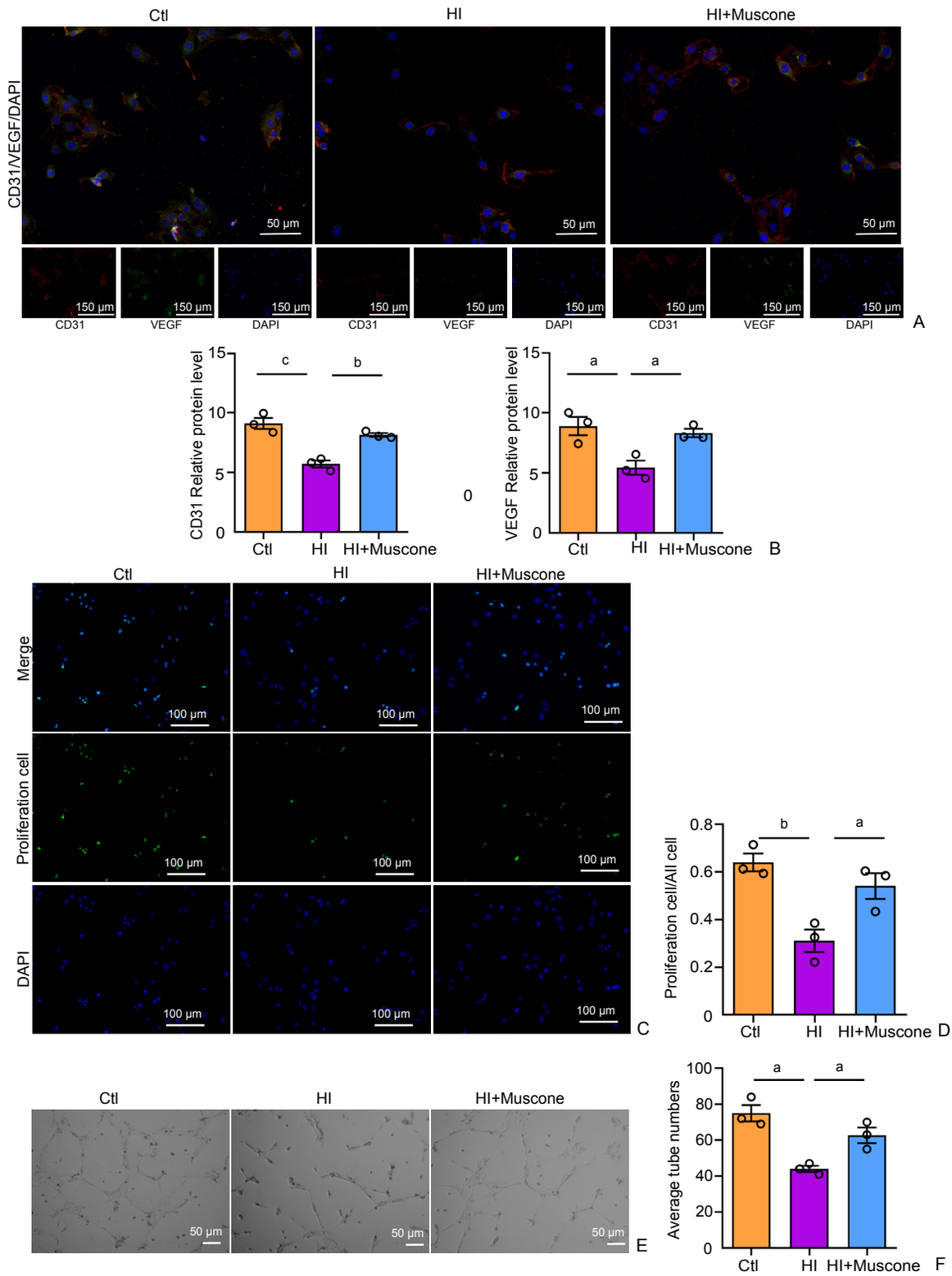
A: Fluorescence double staining for CD31 and VEGF in MCMEC; B: Quantitative analysis of fluorescence double staining for CD31 and VEGF in MCMEC; C: EdU cell proliferation staining in MCMEC; D: Histogram of the proliferation of MCMEC; E: Microscopic images of angiogenesis in MCMEC; F: Histogram of the angiogenesis of MCMEC. MCMEC: Mouse coronary microvascular endothelial cells; SXBXP: Shexiang Baoxin Pills; VEGF: Vascular endothelial growth factor. a: $P<0.05$, b: $P<0.01$, compared with the HI group

图4 SXBXP对MCMEC的VEGF表达及增殖和成管能力的影响

Figure 4 Effects of SXBXP on VEGF expression, proliferation, and angiogenesis of MCMEC

2.5 Muscone能够增加MCMEC的VEGF表达,提高细胞增殖能力和成管能力

检测3组MCMEC中VEGF的表达水平,结果显示HI组中VEGF的表达显著低于Ctl组($P<0.001$),HI+Muscone组中VEGF的表达显著高于HI组($P<0.01$,图5A、B)。增殖实验发现HI组的细胞增殖能力显著低于Ctl组($P<0.01$),而HI+Muscone组的增殖能力显著高于HI组($P<0.05$,图5C、D)。成管实验发现HI组的微血管生成能力显著低于Ctl组($P<0.05$),而HI+Muscone组的成管能力显著高于HI组($P<0.05$,图5E、F)。



A: Fluorescence double staining for CD31 and VEGF in MCMEC; B: Quantitative analysis of fluorescence double staining for CD31 and VEGF in MCMEC; C: EdU cell proliferation staining in MCMEC; D: Histogram of the proliferation of MCMEC. n=3; E: Microscopic images of angiogenesis in MCMEC; F: Histogram of the angiogenesis of MCMEC. MCMEC: Mouse coronary microvascular endothelial cells; SXBXP: Shexiang Baoxin Pills; VEGF: Vascular endothelial growth factor. a: P<0.05, b: P<0.01, c: P<0.01, compared with the normoxia control cells

图 5 Muscone对MCMEC的VEGF表达及增殖和成管能力的影响

Figure 5 Effects of muscone on VEGF expression, proliferation, and angiogenesis of MCMEC

3 讨论

3.1 CMD的传统治疗策略

目前对CMD的治疗策略主要是改善生活方式、管理危险因素、缓解心肌缺血症状[8],其中药物治疗方面主要以他汀类药物、β阻滞剂和钙通道阻滞剂等为主[28],而明确针对冠状动脉微血管的药物仍较为缺乏。有动物研究表明尼可地尔通过PI3K/Akt/eNOS信号通路改善CMD和心脏功能[29],但是对冠状动脉微血密度的影响并不清楚。恩格列净能够增强以糖尿病小鼠为CMD模型的左室收缩功能,改善冠脉功能障碍和增加心脏微血管密度[30],但具体机制也未阐明。

3.2 SXBXP通过促进VEGF表达增强冠状动脉微血管生成改善CMD

研究发现麝香通心滴丸通过降低M1巨噬细胞极化诱导的炎症和内皮功能障碍进而改善CMD[31],但并未明确对冠状动脉微血管结构的影响。本研究发现SXBXP干预能够显著改善CMD小鼠的CFR、MBF和心脏冠状动脉微血管密度,增加VEGF表达,说明SXBXP可能通过促进VEGF表达诱导冠状动脉微血管生成,增加心肌微血流灌注发挥对CMD的改善作用。进一步通过体外实验发现SXBXP促进MCMEC对VEGF的表达,进而改善了心脏微血管内皮细胞的增殖和血管生成能力,而Muscone同样能够诱导MCMEC对VEGF的表达,促进MCMEC的增殖和血管生成,表明在SXBXP中Muscone作为主要的活性物质在促进血管生成方面发挥重要作用。这与恩格列净改善CMD的表型一致,但本研究中发现CMD小鼠并未出现左心室收缩功能改变。鉴于在多种疾病中,微血管密度稀疏通常先于收缩功能障碍出现[32],提示早期SXBXP干预可能通过保持充足的微血管密度来延缓疾病进展。

3.3 VEGF可能是Muscone促进微血管形成并改善CMD的关键靶点

心脏微血管密度稀疏是CMD最重要的表型之一,是引起心肌缺血的病理生理学变化[33-34]。本研究发现SXBXP可促进心脏微血管生成从而使微血管密度增加,且心脏微血管密度与CFR、MBF存在正相关性关系,提示微血管密度增加可能是冠状动脉血流储备和心肌血流灌注的结构基础。先前研究表明SXBXP可缓解稳定型心绞痛以及心肌缺血引起的胸痛[35],但这项临床研究主要聚焦在临床疗效和安全性分析,并未明确具体机制。同样有研究显示SXBXP可能促进心脏血管生成,但这些研究主要基于心内膜细胞或人脐静脉内皮细胞的研究[9,36],而非心脏微血管内皮细胞。也有研究发现SXBXP可以改善低氧诱导的微血管生成抑制,但这项研究中并未检测心肌的缺血情况[16]。此外,多项研究证实了SXBXP在CAD小鼠或细胞模型中促进血管生成的潜力[15,37],麝香作为SXBXP中的重要成分可能发挥着重要作用。既往研究显示Muscone作为麝香最重要的单体,体外研究发现Muscone对心肌缺血/再灌注引起的损伤具有保护作用[38],但对心脏微血管生成的影响机制仍不清楚。在血管生成过程中,VEGF依赖性血管生成是核心机制[39-40]。SXBXP干预CMD小鼠显著提高了心脏血管内皮细胞中VEGF的表达水平,且与心脏微血管密度的改善密切相关。在体外Muscone诱导MCMEC对VEGF表达,促进其增殖和血管生成,表明VEGF可能是Muscone促进微血管形成并改善CMD的关键靶点。

3.4 总结

本研究结果表明:Muscone作为SXBXP的主要活性物质,通过增加VEGF表达促进心脏微血管生成,逆转微血管稀疏,增加心肌血流灌注,为Muscone用于CMD的治疗提供了实验证据。但Muscone对VEGF表达和相关表型的调节作用机制需要进一步研究,为精准干预CMD提供潜在的新型干预靶点和方案。

利益冲突 本文所有作者声明不存在利益冲突

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