

黄芪甲苷通过下调 miR-327 缓解缺氧对脑微血管内皮细胞凋亡及炎症反应的影响

刘丹¹, 于广周², 宋景贵^{1*} (1. 驻马店市中心医院神经内三科, 河南 驻马店 463000; 2. 新乡医学院第二附属医院神经内科, 河南 新乡 453000)

摘要:目的 探讨黄芪甲苷对缺氧处理的脑微血管内皮细胞(BMECs)损伤的影响及分子机制。方法 体外培养BMECs,将其分为对照组、模型组、低、中、高剂量黄芪甲苷组、高剂量黄芪甲苷+miR-NC、高剂量黄芪甲苷+miR-327;四甲基偶氮唑盐比色法(MTT)检测细胞活力;流式细胞术检测细胞凋亡;蛋白质印迹(western blot)法检测蛋白表达;试剂盒检测BMECs损伤标志物,包括乳酸脱氢酶(LDH)、丙二醛(MDA)、超氧化物歧化酶(SOD)和谷胱甘肽过氧化物酶(GSH-Px);酶联免疫吸附实验(ELISA)检测白细胞介素-6(IL-6)、白细胞介素-1 β (IL-1 β)和肿瘤坏死因子- α (TNF- α);实时荧光定量PCR(RT-qPCR)检测miR-327的表达水平。结果 与对照组比较,缺氧处理9 h与12 h处,BMECs存活率显著降低($P < 0.05$)。与对照组相比,模型组细胞凋亡率显著升高,Bcl-2表达水平显著降低,Bax表达水平显著升高($P < 0.05$);与模型组相比,低、中、高剂量黄芪甲苷组细胞凋亡率均显著降低,Bcl-2表达水平升高,Bax表达水平降低,且呈剂量依赖性($P < 0.05$)。与对照组相比,模型组LDH、MDA、IL-6、IL-1 β 和TNF- α 表达水平显著升高,SOD和GSH-Px表达水平显著降低($P < 0.05$);与模型组相比,低、中、高剂量黄芪甲苷组LDH、MDA、IL-6、IL-1 β 和TNF- α 表达水平显著降低,SOD和GSH-Px表达水平显著升高,且呈剂量依赖性($P < 0.05$)。与对照组相比,模型组miR-327表达水平显著升高($P < 0.05$);与模型组相比,低、中、高剂量黄芪甲苷组miR-327表达水平显著降低,且呈剂量依赖性($P < 0.05$)。过表达miR-327逆转了黄芪甲苷对缺氧处理BMECs凋亡和炎症反应的影响。结论 黄芪甲苷可能通过下调miR-327表达缓解缺氧对BMECs凋亡及炎症反应的影响。

关键词:黄芪甲苷;miR-327;凋亡;炎症反应

doi:10.11669/cpj.2021.19.007 中图分类号:R965 文献标志码:A 文章编号:1001-2494(2021)19-1571-06

Astragaloside IV Alleviates the Effects of Hypoxia on Brain Microvascular Endothelial Cells Apoptosis and Inflammation by Down-Regulating miR-327

LIU Dan¹, YU Guang-zhou², SONG Jing-gui^{1*} (1. Department of Neurology, Zhumadian Central Hospital, Zhumadian 463000, China; 2. Department of Neurology, the Second Affiliated Hospital of Xinxiang Medical College, Xinxiang 453000, China)

ABSTRACT: OBJECTIVE To explore the effect of astragaloside IV on the damage of brain microvascular endothelial cells (BMECs) treated with hypoxia and its molecular mechanism. **METHODS** BMECs were cultured *in vitro* and divided into control group, model group, low, medium and high dose astragaloside IV groups, high dose astragaloside IV + miR-NC group, and high dose astragaloside IV + miR-327 group. The tetramethylazolate colorimetric method (MTT) was used to detect cell activity. Flow cytometry was used to detect cell apoptosis. Western blot was used to detect protein expression. The kits detected damage markers of BMECs, including lactate dehydrogenase (LDH), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX). ELISA was used to evaluate the levels of interleukin-6 (IL-6), interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). Real-time fluorescence quantitative PCR (RT-qPCR) was used to detect the expression of circDONSON. **RESULTS** Compared with the control group, the survival rate of BMECs decreased significantly at 9 h and 12 h after hypoxia treatment ($P < 0.05$). Compared with the control group, the apoptosis rate of the model group was significantly increased, Bcl-2 expression level was significantly decreased, and Bax expression level was significantly increased ($P < 0.05$). Compared with the model group, the apoptosis rates of the low, medium and high dose astragaloside IV groups were significantly reduced, the expression level of Bcl-2 was increased, and the expression level of Bax was decreased in a dose-dependent manner ($P < 0.05$). Compared with the control group, LDH, MDA, IL-6, IL-1 β and TNF- α expression levels in the model group were significantly increased, while SOD and GSH-

基金项目:河南省医学科技攻关计划省部共建项目资助(SB201901063)

作者简介:刘丹,女,硕士研究生,副主任医师 研究方向:脑血管病 * **通讯作者:**宋景贵,男,主任医师,博士生导师 研究方向:脑血管病 Tel:(0396)2726128

PX expression levels were significantly decreased ($P < 0.05$). Compared with the model group, the expression levels of LDH, MDA, IL-6, IL-1 β and TNF- α in the low, medium and high dose astragaloside IV groups were significantly decreased, and the expression levels of SOD and GSH-PX were significantly increased in a dose-dependent manner ($P < 0.05$). Compared with the control group, miR-327 expression level in the model group was significantly increased ($P < 0.05$). Compared with the model group, the expression level of miR-327 in the low, medium and high dose astragaloside IV groups was significantly reduced dose-dependently ($P < 0.05$). The overexpression of miR-327 reversed the effects of astragaloside IV on the apoptosis and inflammatory response of hypoxia treated BMECs. **CONCLUSION** Astragaloside IV may alleviate the effects of hypoxia on BMECs apoptosis and inflammation by down-regulating the expression of miR-327.

KEY WORDS: astragaloside IV; miR-327; apoptosis; inflammation

缺血性脑卒中是最常见的脑血管疾病,具有发病率高、发病快且迅速、死亡率高等特点,且其发病率仍呈上升趋势^[1-3]。脑缺血损伤是缺血性脑卒中的基本病理生理基础,其发病机制复杂,涉及能量衰竭、酸中毒、线粒体损伤、氧化应激和炎症反应等,最终导致细胞坏死或凋亡^[4-6]。低氧微环境参与多种疾病的发病,并能够调控细胞增殖、分化、凋亡和血管生成等过程^[7]。缺氧在脑血管疾病中较为常见,并造成脑损伤,尤其容易造成内皮细胞的损伤^[8]。中草药在脑卒中及其相关疾病的临床应用方面历史悠久,黄芪在脑缺血的中医治疗中十分重要^[9-10]。黄芪甲苷是从黄芪中提取的一种有效成分,具有抗炎、抗氧化、抗凋亡等作用^[11]。黄芪甲苷能显著减轻缺血再灌注对大鼠神经功能的损伤,减少脑梗死和神经元凋亡^[12]。黄芪甲苷可能用过调控凋亡相关基因缓解缺糖缺氧/复糖复氧对大鼠脑微血管内皮细胞及胶质细胞的损伤^[13]。但黄芪甲苷缓解脑损伤的作用机制仍不清楚。微小RNA(miRNA)是一类长度仅为18~25 bp的非编码RNA,其参与缺血性脑损伤的发病机制,是中风的潜在治疗靶点。研究报道,miR-327在脑缺血再灌注期间表达上调^[14]。然而黄芪甲苷对缺氧诱导的脑微血管内皮细胞(BMECs)损伤的影响及其机制是否与miR-327有关尚未完全明确。因此,本实验旨在研究黄芪甲苷是否通过调控miR-327影响低氧诱导的BMECs损伤。

1 材料与方法

1.1 材料

本研究经我院伦理委员会批准,并尽力减少对动物的不适。雄性C57BL/6小鼠(8~10周,20~25 g)购自SLRC实验室(中国上海)。大鼠BMECs按照Diglio获取^[15],原代BMECs培养在含10%胎牛血清(FBS)、75 $\mu\text{g} \cdot \text{mL}^{-1}$ 内皮细胞生长补充剂和0.5 $\text{mg} \cdot \text{mL}^{-1}$ 肝素的DMEM培养基中。当BMECs

汇合率为90%时传代,细胞在37 $^{\circ}\text{C}$ 的5% CO_2 培养箱中保存。

1.2 试剂

黄芪甲苷(纯度 $\geq 98\%$)(南京石洲生物科技有限公司);DMEM培养基、胎牛血清(美国Hyclone公司);MTT试剂盒(上海晶抗生物工程有限公司);Annexin V-FITC/PI凋亡检测试剂盒(北京凯瑞基生物科技有限公司);RIPA蛋白裂解液、二辛可宁酸(bicinchoninic acid, BCA)试剂盒(上海研谨生物科技有限公司);LDH、SOD、MDA、GSH-Px检测试剂盒(南京建城生物工程有限公司);ELISA试剂盒(北京天根生物技术公司);Trizol试剂、反转录试剂盒、荧光定量试剂盒(日本Takara公司);miR-NC、miR-327(上海吉玛制药技术有限公司);Lipofectamine 2000(上海信帆生物科技有限公司)。

1.3 缺氧处理与分组

取第3代生长状态良好的BMECs,细胞在氧含量5%左右的培养箱中培养12 h,记为模型组,对照组氧含量正常。分别用浓度为10、20、30 $\mu\text{mol} \cdot \text{L}^{-1}$ 黄芪甲苷和低氧处理BMECs,记为低、中、高剂量黄芪甲苷组。将miR-NC、miR-327转染至BMECs后,用30 $\mu\text{mol} \cdot \text{L}^{-1}$ 黄芪甲苷和低氧处理,记为高剂量黄芪甲苷+miR-NC组、高剂量黄芪甲苷+miR-327组。各组转染均按照Lipofectamine2000试剂盒说明进行。

1.4 细胞增殖

将对照组缺氧处理3、6、9、12 h的BMECs($2 \times$ 每毫升 10^5 个)接种于96孔板(每孔100 μL),培养24 h,加入MTT溶液(每孔20 μL),室温孵育4 h,弃上清,加入DMSO(每孔150 μL),室温振荡孵育5 min,酶标仪检测490 nm处的吸光度(OD值),即代表细胞活性。

1.5 Annexin V检测细胞凋亡

按照Annexin V-FITC/PI试剂盒说明检测各组

BMECs 凋亡率,收集各组细胞并用 PBS 漂洗 2 次,结合缓冲液重悬后,加入 Annexin V-FITC、碘化丙啶 (PI) 各 5 μL ,37 $^{\circ}\text{C}$ 孵育 15 min 后,用流式细胞仪检测细胞凋亡。

1.6 蛋白质印迹 (Western blot) 法检测蛋白表达

提取各组细胞总蛋白,用 BCA 试剂盒进行定量。各组蛋白上样量 60 μg ,进行 SDS-PAGE 后转移至 PVDF 上,用 5% 脱脂牛奶室温封闭,然后加入一抗 4 $^{\circ}\text{C}$ 孵育过夜,PBS 洗涤 3 次,加入二抗室温孵育 2 h,PBS 洗涤 3 次,暗室中曝光显影,定影,用 Quantity One 软件检测各组蛋白条带灰度值,以目的条带和 GAPDH 条带的比值作为蛋白表达水平。

1.7 LDH、MDA、SOD 和 GSH-Px 的检测

取各组对数生长期的 BMECs 接种于 96 孔板,接种密度为每毫升 1×10^5 个,每孔 100 μL 。孵育 24 h 后,细胞按“1.3”项下所述进行分组。采用黄芪甲苷 (10、20、30 $\mu\text{mol} \cdot \text{L}^{-1}$) 和低氧处理 12 h,收集各组上清液,上清液中 LDH、MDA、SOD 和 GSH-Px 的水平按照试剂盒说明进行测定。

1.8 酶联免疫吸附试验 (ELISA) 检测 IL-6、IL-1 β 和 TNF- α 的表达

按照 ELISA 试剂盒说明检测 BMECs 中 IL-6、IL-1 β 和 TNF- α 的表达水平。

1.9 实时荧光定量 PCR (RT-qPCR)

提取各组 BMECs 的总 RNA,反转录成 cDNA,按试剂盒说明进行 PCR,检测各组 miR-327 的水平。miR-327 上游引物序列: 5'-TGCGCCCTT-GAGGGGCATG-3',下游引物序列: 5'-CAGTG-CAGGGTCCGAGGTATT-3'; U6 上游引物序列: 5'-CGCTTCGGCAGCACATATAC-3',下游引物序列: 5'-AAATATGGAACGCTTCACGA-3';引物由上海生工生物工程公司合成。

1.10 统计学处理

采用 SPSS21.0 统计学软件分析数据,计量资料以 ($\bar{x} \pm s$) 表示且均符合正态分布,两组间比较采用独立样本 t 检验,多组间比较采用单因素方差分析,以 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 缺氧对 BMECs 活力的影响

与 Control 组相比,随着缺氧处理时间的增加,BMECs 存活率下降,且在缺氧处理 9 h 与 12 h 处,存活率显著降低 ($P < 0.05$) (表 1)。

表 1 缺氧对脑微血管内皮细胞 (BMECs) 存活率的影响。 $n = 9, \bar{x} \pm s$

Tab.1 Effects of hypoxia on the survival rate of brain microvascular endothelial cells (BMECs). $n = 9, \bar{x} \pm s$

Groups	Survival rate/%
Control	100.00 \pm 4.18
3 h	96.29 \pm 3.73
6 h	89.16 \pm 2.61
9 h	79.45 \pm 2.21 ¹⁾
12 h	67.17 \pm 1.36 ¹⁾
<i>F</i>	19.713
<i>P</i>	0.000

注:与对照组相比,¹⁾ $P < 0.05$

Note: ¹⁾ $P < 0.05$, compared with the control group

2.2 黄芪甲苷对缺氧处理 BMECs 凋亡的影响

与 Control 组相比,Model 组细胞凋亡率显著升高,Bcl-2 表达水平显著降低,Bax 表达水平显著升高 ($P < 0.05$);与 Model 组相比,10、20、30 $\mu\text{mol} \cdot \text{L}^{-1}$ 黄芪甲苷组细胞凋亡率均显著降低,Bcl-2 表达水平升高,Bax 表达水平降低,且呈剂量依赖性 ($P < 0.05$) (图 1,表 2)。

2.3 黄芪甲苷对缺氧处理 BMECs 中 LDH、MDA、SOD、GSH-Px、IL-6、IL-1 β 和 TNF- α 的影响

与 Control 组相比,Model 组 LDH、MDA、IL-6、IL-1 β 和 TNF- α 表达水平显著升高,SOD 和 GSH-Px 表达水平显著降低 ($P < 0.05$);与 Model 组相比,10、20、30 $\mu\text{mol} \cdot \text{L}^{-1}$ 黄芪甲苷组 LDH、MDA、IL-6、IL-1 β 和 TNF- α 表达水平显著降低,SOD 和 GSH-Px 表达水平显著升高 ($P < 0.05$) (表 3)。

2.4 黄芪甲苷对缺氧处理 BMECs 中 miR-327 的影响

与 Control 组相比,Model 组 miR-327 表达水平显著升高 ($P < 0.05$);与 Model 组相比,10、20、30 $\mu\text{mol} \cdot \text{L}^{-1}$ 黄芪甲苷组 miR-327 表达水平显著降低 ($P < 0.05$) (表 4)。

2.5 过表达 miR-327 逆转了黄芪甲苷对缺氧处理 BMECs 凋亡的影响

与 30 $\mu\text{mol} \cdot \text{L}^{-1}$ + miR-NC 组相比,30 $\mu\text{mol} \cdot \text{L}^{-1}$ + miR-327 组的 miR-327 表达水平和细胞凋亡率显著升高,Bcl-2 表达水平显著降低,Bax 表达水平显著升高 ($P < 0.05$) (图 2,表 5)。

2.6 过表达 miR-327 逆转了黄芪甲苷对缺氧处理 BMECs 中 LDH、MDA、SOD、GSH-Px、IL-6、IL-1 β 和 TNF- α 的影响

与 30 $\mu\text{mol} \cdot \text{L}^{-1}$ + miR-NC 组相比,30 $\mu\text{mol} \cdot \text{L}^{-1}$ + miR-327 组 LDH、MDA、IL-6、IL-1 β 和 TNF- α

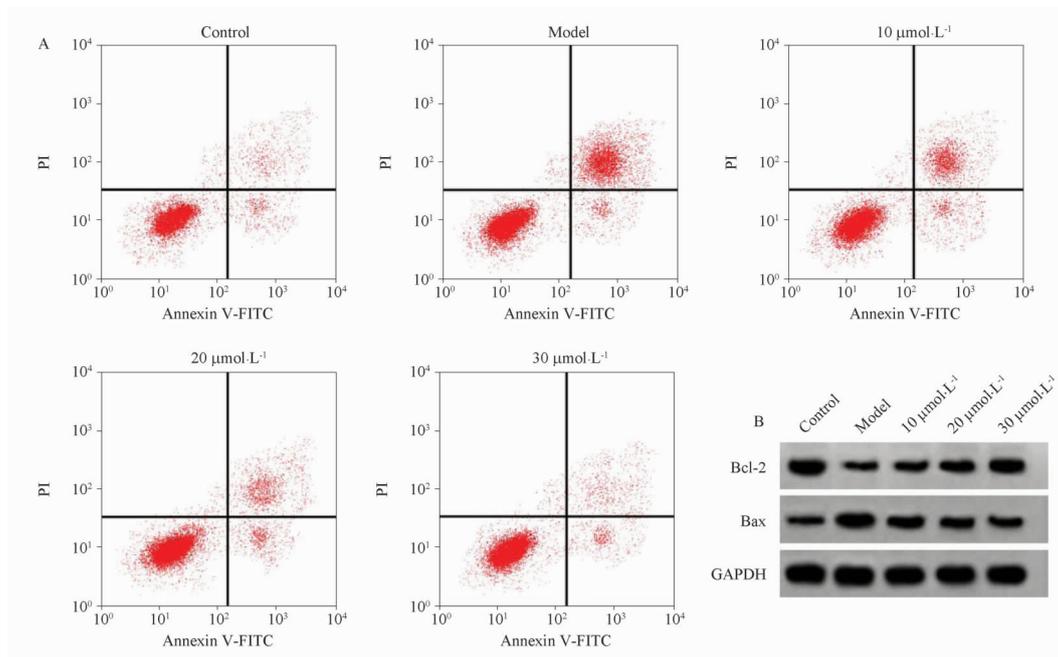


图1 黄芪甲苷对缺氧处理 BMECs 凋亡的影响

A - 细胞凋亡流式图; B - 细胞凋亡相关蛋白表达

Fig. 1 Effects of astragaloside IV on apoptosis of BMECs treated with hypoxia

A - cell apoptosis; B - expression of apoptosis-related proteins

表2 黄芪甲苷对缺氧处理 BMECs 凋亡的影响. $n=9, \bar{x} \pm s$

Tab. 2 Effects of astragaloside IV on apoptosis of BMECs treated with hypoxia. $n=9, \bar{x} \pm s$

Groups	Apoptosis rate/%	Bcl-2	Bax
Control	3.66 ± 0.33	0.85 ± 0.06	0.19 ± 0.01
Model	38.89 ± 2.47 ¹⁾	0.25 ± 0.01 ¹⁾	0.79 ± 0.03 ¹⁾
10 μmol · L ⁻¹	29.53 ± 2.16 ²⁾	0.39 ± 0.02 ²⁾	0.66 ± 0.03 ²⁾
20 μmol · L ⁻¹	19.26 ± 1.94 ²⁾	0.58 ± 0.04 ²⁾	0.51 ± 0.04 ²⁾
30 μmol · L ⁻¹	5.46 ± 0.64 ²⁾	0.78 ± 0.05 ²⁾	0.28 ± 0.03 ²⁾
F	76.769	40.346	69.857
P	0.000	0.000	0.000

注:与对照组相比, ¹⁾ $P < 0.05$;与模型组相比, ²⁾ $P < 0.05$

Note: ¹⁾ $P < 0.05$, compared with the control group; ²⁾ $P < 0.05$, compared with the model group

表达水平显著升高, SOD 和 GSH-Px 表达水平显著降低($P < 0.05$) (表 6)。

3 讨论

BMECs 是一层由连接和锚定依赖的细胞组成的薄层,其功能受化学、物理和机械刺激的影响^[16]。BMECs 功能障碍可引发脑组织损伤,例如中风、创伤性脑损伤和神经退行性疾病,这些损伤通过反馈回路加剧 BMECs 功能障碍^[17-18]。乳酸脱氢酶(LDH)广泛分布于神经元和胶质细胞中,脑内含量

丰富,在脑缺血损伤时,LDH 释放到血液中,从而增加乳酸的积累^[19]。丙二醛(MDA)是多不饱和脂肪酸脂质过氧化的主要产物,常用来衡量氧化应激水平^[20]。超氧化物歧化酶(SOD)和谷胱甘肽过氧化物酶(GSH-Px)是有效的天然抗氧化酶,可通过清除细胞内活性氧来发挥生物保护作用,SOD 和 GSH-Px 活性的增加可缓解脑缺血所造成的氧化应激损伤^[21-22]。IL-6、IL-1 β 和 TNF- α 等是脑损伤加重过程中重要的炎性细胞因子标志物。黄芪甲苷通过调控 SOD 活性、LDH 和 MDA 水平缓解 LPS 诱导的 MLE-12 细胞损伤,发挥其对急性呼吸窘迫综合征的治疗作用^[23]。黄芪甲苷预处理提高了细胞存活率、SOD、CAT、和 GSH-Px 活性,降低了 MDA 水平和凋亡率,缓解了 H₂O₂ 诱导的小肠上皮细胞损伤^[24]。与上述抗炎结果一致,本研究发现黄芪甲苷以剂量依赖方式降低缺氧所致的 BMECs 凋亡和炎症反应,显著降低 Bax、LDH、MDA、IL-6、IL-1 β 和 TNF- α 水平,显著提高 Bcl-2、SOD 和 GSH-Px 水平,与功能分析结果相一致,这表明,黄芪甲苷可降低缺氧所致的 BMECs 损伤,具有具有抗炎、抗氧化、抗凋亡等作用。

近年来,多项研究证实黄芪甲苷通过调控 miR 表达缓解各种细胞损伤。如黄芪甲苷通过 miR-210

表3 黄芪甲苷对缺氧处理 BMECs 中 LDH、MDA、SOD、GSH-Px、IL-6、IL-1 β 和 TNF- α 表达的影响. $n=9, \bar{x} \pm s$

Tab.3 Effects of astragaloside IV on expressions of LDH, MDA, SOD, GSH-Px, IL-6, IL-1 β and TNF- α in hypoxic-treated BMECs. $n=9, \bar{x} \pm s$

Groups	concentration LDH /U · g ⁻¹	concentration MDA /nmol · mg ⁻¹	concentration SOD /U · mg ⁻¹	concentration GSH-Px /U · mg ⁻¹	ρ (IL-6) /pg · mL ⁻¹	ρ (IL-1 β) /pg · mL ⁻¹	ρ (TNF- α) /pg · mL ⁻¹
Control	87.85 ± 4.22	0.55 ± 0.03	52.66 ± 2.96	112.74 ± 4.74	31.83 ± 2.53	49.37 ± 2.86	96.41 ± 3.75
Model	289.72 ± 12.37 ¹⁾	1.79 ± 0.07 ¹⁾	19.27 ± 1.30 ¹⁾	58.11 ± 1.81 ¹⁾	127.75 ± 6.34 ¹⁾	145.36 ± 6.81 ¹⁾	223.12 ± 8.97 ¹⁾
10 μ mol · L ⁻¹	265.17 ± 8.26 ²⁾	1.52 ± 0.05 ²⁾	25.65 ± 1.58 ²⁾	68.86 ± 2.23 ²⁾	109.46 ± 3.66 ²⁾	121.81 ± 3.75 ²⁾	186.87 ± 7.45 ²⁾
20 μ mol · L ⁻¹	205.46 ± 6.98 ²⁾	1.13 ± 0.06 ²⁾	39.12 ± 2.18 ²⁾	85.35 ± 2.84 ²⁾	86.35 ± 3.71 ²⁾	98.15 ± 4.37 ²⁾	153.56 ± 4.67 ²⁾
30 μ mol · L ⁻¹	103.37 ± 6.12 ²⁾	0.69 ± 0.05 ²⁾	45.51 ± 2.73 ²⁾	97.31 ± 3.50 ²⁾	52.73 ± 2.39 ²⁾	62.96 ± 2.76 ²⁾	113.31 ± 4.61 ²⁾
F	161.493	102.797	37.877	46.653	98.077	83.454	70.076
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

注:与对照组相比,¹⁾ $P < 0.05$;与模型组相比,²⁾ $P < 0.05$

Note: ¹⁾ $P < 0.05$, compared with the control group; ²⁾ $P < 0.05$, compared with the model group

表4 黄芪甲苷对缺氧处理 BMECs 中 miR-327 的影响. $n=9, \bar{x} \pm s$

Tab.4 Effects of astragaloside IV on miR-327 in hypoxic-treated BMECs. $n=9, \bar{x} \pm s$

Groups	miR-327
Control	1.01 ± 0.06
Model	3.18 ± 0.16 ¹⁾
10 μ mol · L ⁻¹	2.63 ± 0.11 ²⁾
20 μ mol · L ⁻¹	1.97 ± 0.12 ²⁾
30 μ mol · L ⁻¹	1.31 ± 0.09 ²⁾
F	65.776
P	0.000

注:与对照组相比,¹⁾ $P < 0.05$;与模型组相比,²⁾ $P < 0.05$

Note: ¹⁾ $P < 0.05$, compared with the control group; ²⁾ $P < 0.05$, compared with the model group

表5 过表达 miR-327 逆转了黄芪甲苷对缺氧处理 BMECs 凋亡的影响. $n=9, \bar{x} \pm s$

Tab.5 Overexpression of miR-327 reversed the effect of astragaloside IV on the apoptosis of hypoxic-treated BMECs. $n=9, \bar{x} \pm s$

Groups	miR-327	Apoptosis rate/%	Bcl-2	Bax
30 μ mol · L ⁻¹ + miR-NC	1.02 ± 0.05	6.73 ± 0.42	0.78 ± 0.04	0.29 ± 0.01
30 μ mol · L ⁻¹ + miR-327	2.49 ± 0.12 ¹⁾	27.16 ± 1.76 ¹⁾	0.46 ± 0.03 ¹⁾	0.58 ± 0.02 ¹⁾
t	11.678	11.322	6.548	15.092
P	0.000	0.000	0.003	0.000

注:与 30 μ mol · L⁻¹ + miR-NC 组比较,¹⁾ $P < 0.05$

Note: ¹⁾ $P < 0.05$, compared with the 30 μ mol · L⁻¹ + miR-NC group

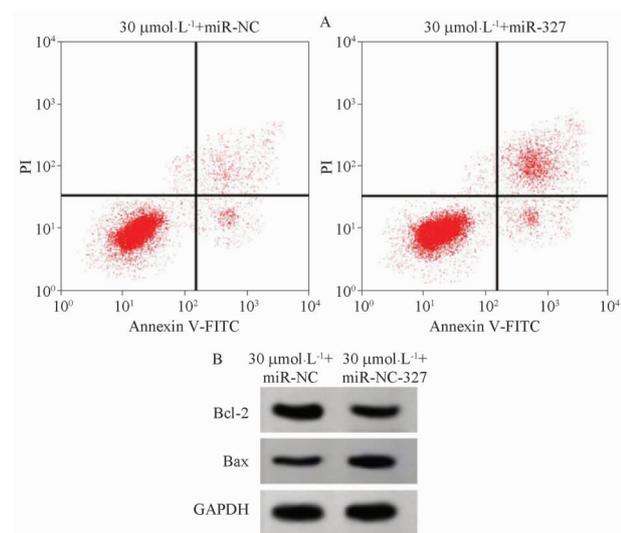


图2 过表达 miR-327 逆转了黄芪甲苷对缺氧处理 BMECs 凋亡的影响

A-细胞凋亡流式图; B-细胞凋亡相关蛋白表达

Fig.2 Overexpression of miR-327 reversed the effect of astragaloside IV on the apoptosis of hypoxic-treated BMECs

A - cell apoptosis; B - expression of apoptosis-related proteins

激活 HIF/VEGF/Notch 信号通路来缩小梗死面积和促进细胞增殖和导管形成,以缓解缺血性脑损伤^[25]。黄芪甲苷通过抑制 miR-124 表达来减轻缺氧诱导的 PC-12 细胞损伤^[26]。黄芪甲苷通过下调 miR-23a 和 miR-92a 表达缓解缺氧诱导对大鼠心肌细胞的损伤^[27]。本研究发现,模型组中 miR-327 水平显著升高,而黄芪甲苷处理可显著降低 miR-327 水平,提示黄芪甲苷可能通过调控 miR-327 表达在缺血性脑卒中中发挥抗炎、抗氧化、抗凋亡等作用。此外,上调 miR-327 表达显著降低黄芪甲苷对细胞凋亡和炎症反应的影响,这进一步说明,上调 miR-327 可能是黄芪甲苷在缺血性脑卒中中发挥抗炎、抗氧化、抗凋亡等作用的重要途径。

综上,黄芪甲苷可能通过下调 miR-327 表达缓解缺氧所致的 BMECs 凋亡和炎症反应,这丰富了中药化合物与 miR 之间的调控关系,有助于了解缺血性脑卒中细胞损伤的病理过程,为黄芪甲苷治疗缺血性脑卒中提供理论依据。

表 6 过表达 miR-327 逆转了黄芪甲苷对缺氧处理 BMECs 中 LDH、MDA、SOD、GSH-Px、IL-6、IL-1 β 和 TNF- α 的影响. $n=9, \bar{x} \pm s$
Tab. 6 Overexpression of miR-327 reversed the effects of astragaloside IV on LDH, MDA, SOD, GSH-Px, IL-6, IL-1 β and TNF- α in hypoxic-treated BMECs. $n=9, \bar{x} \pm s$

Group	concentration	concentration	concentration	concentration	ρ (IL-6)	ρ (IL-1 β)	ρ (TNF- α)
	LDH/U \cdot g $^{-1}$	MDA/nmol \cdot mg $^{-1}$	SOD/U \cdot mg $^{-1}$	GSH-Px/U \cdot mg $^{-1}$	/pg \cdot mL $^{-1}$	/pg \cdot mL $^{-1}$	/pg \cdot mL $^{-1}$
30 μ mol \cdot L $^{-1}$ + miR-NC	108.68 \pm 4.64	0.73 \pm 0.04	48.25 \pm 1.22	95.39 \pm 2.34	45.29 \pm 2.10	55.89 \pm 3.11	113.78 \pm 4.25
30 μ mol \cdot L $^{-1}$ + miR-327	245.58 \pm 8.42 ¹⁾	1.39 \pm 0.05 ¹⁾	33.11 \pm 0.69 ¹⁾	74.25 \pm 1.07 ¹⁾	97.12 \pm 3.85 ¹⁾	109.47 \pm 4.50 ¹⁾	175.23 \pm 5.26 ¹⁾
<i>t</i>	14.236	10.957	10.822	8.221	11.819	9.797	9.081
<i>P</i>	0.000	0.000	0.000	0.001	0.000	0.001	0.001

注:与 30 μ mol \cdot L $^{-1}$ + miR-NC 组比较,¹⁾ $P < 0.05$

Note:¹⁾ $P < 0.05$, compared with the 30 μ mol \cdot L $^{-1}$ + miR-NC group

REFERENCES

- [1] RANDOLPH S A. Ischemic stroke[J]. *Workplace Health Saf*, 2016, 64(9): 444.
- [2] STACK C A, COLE J W. Ischemic stroke in young adults[J]. *Curr Opin Cardiol*, 2018, 33(6): 594-604.
- [3] CABRAL N L, NAGEL V, CONFORTO A B, et al. Five-year survival, disability, and recurrence after first-ever stroke in a middle-income country: a population-based study in Joinville, Brazil[J]. *Int J Stroke*, 2018, 13(7):725-733.
- [4] CARELLI V, CHAN D C. Mitochondrial DNA: impacting central and peripheral nervous systems[J]. *Neuron*, 2014, 84(6): 1126-1142.
- [5] SUN D, WANG W, WANG X, et al. bFGF plays a neuroprotective role by suppressing excessive autophagy and apoptosis after transient global cerebral ischemia in rats[J]. *Cell Death Dis*, 2018, 9(2):172-185.
- [6] LALU M M, FERGUSSON D A, CHENG W, et al. Identifying stroke therapeutics from preclinical models: a protocol for a novel application of network meta-analysis[J]. *F1000 Res*, 2019, 8: 11-26.
- [7] LI Y, TIAN Z, XU M L, et al. Effect and mechanism of hypoxic, micro-environment on the proliferation and apoptosis of rat brain vascular endothelial cells[J]. *Prog Mod Biomed* (现代生物医学进展), 2019, 19(02):222-226,257.
- [8] LIU X, DENG F, YU Z, et al. Inhibition of endothelin a receptor protects brain microvascular endothelial cells against hypoxia-induced injury[J]. *Int J Mol Med*, 2014, 34(1):313-320.
- [9] SETO SW, CHANG D, JENKINS A, et al. Angiogenesis in ischemic stroke and angiogenic effects of chinese herbal medicine [J]. *J Clin Med*, 2016, 5(6):56-71.
- [10] LI X X, QU L, DONG Y Z, et al. A review of recent research progress on the astragalus genus[J]. *Molecules*, 2014, 19(11), 18850-18880.
- [11] SONG M T, RUAN J, ZHANG R Y, et al. Astragaloside IV ameliorates neuroinflammation-induced depressive-like behaviors in mice via the PPAR γ /NF- κ B/NLRP3 inflammasome axis[J]. *Acta Pharmacol Sin*, 2018, 39(10): 1559-1570.
- [12] YIN F, ZHOU H, FANG Y, et al. Astragaloside IV alleviates ischemia reperfusion-induced apoptosis by inhibiting the activation of key factors in death receptor pathway and mitochondrial pathway[J]. *J Ethnopharmacol*, 2020, 248: 112319-112342.
- [13] ZHAO Q H. Protection mechanisms of endothelial cells and glial cells astragaloside to OGD complex sugar reoxygenation brain microvessels[D]. Henan: Henan University, 2015.
- [14] YIN K J, HAMBLIN M, CHEN Y E. Angiogenesis-regulating microRNAs and ischemic stroke [J]. *Curr Vasc Pharmacol*, 2015, 13(3):352-365.
- [15] DIGLIO C A, GRAMMAS P, GIACOMELLI F, et al. Primary culture of rat cerebral microvascular endothelial cells. Isolation, growth, and characterization[J]. *Lab Invest*, 1982, 46(6):554-563.
- [16] YU Q J, TAO H, WANG X, et al. Targeting brain microvascular endothelial cells: a therapeutic approach to neuroprotection against stroke [J]. *Neural Regen Res*, 2015, 10(11): 1882-1891.
- [17] KRUEGER M, BECHMANN I, IMMIG K, et al. Blood-brain barrier breakdown involves four distinct stages of vascular damage in various models of experimental focal cerebral ischemia[J]. *J Cereb Blood Flow Metab*, 2014, 35(2):292-303.
- [18] LIU Y, PAN Q, ZHAO Y, et al. MicroRNA-155 regulates ROS production NO generation apoptosis and multiple functions of human brain microvessel endothelial cells under physiological and pathological conditions[J]. *J Cell Biochem*, 2015, 116(12): 2870-2881.
- [19] CHEN Z X, XU Q Q, SHAN C S, et al. Borneol for regulating the permeability of the blood - brain barrier in experimental ischemic stroke: preclinical evidence and possible mechanism[J]. *Oxid Med Cell Longev*, 2019: 2936737-2936752.
- [20] TSIKAS D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges[J]. *Anal Biochem*, 2017, 524:13-30.
- [21] MANZANERO S, SANTRO T, ARUMUGAM T V. Neuronal oxidative stress in acute ischemic stroke: sources and contribution to cell injury[J]. *Neurochem Int*, 2013, 62(5):712-718.
- [22] SUN M S, JIN H, SUN X, et al. Free radical damage in ischemia-reperfusion injury: an obstacle in acute ischemic stroke after revascularization therapy [J]. *Oxid Med Cell Longev*, 2018: 3804979-3804996.
- [23] LIU B, ZHAO H, WANG Y, et al. Astragaloside IV attenuates lipopolysaccharides-induced pulmonary epithelial cell injury through inhibiting autophagy[J]. *Pharmacology*, 2020, 105(1-2):90-101.
- [24] WANG Y, JIANG F, CHENG H, et al. Astragaloside IV protects against oxidative stress in calf small intestine epithelial cells via NFE2L2-antioxidant response element signaling [J]. *Int J Mol Sci*, 2019, 20(24):6131-6145.
- [25] LIANG C, NI G X, SHI X L, et al. Astragaloside IV regulates the HIF/VEGF/Notch signaling pathway through miRNA-210 to promote angiogenesis after ischemic stroke [J]. *Restor Neurol Neurosci*, 2020, 38(3):271-282.
- [26] YU W, LV Z, ZHANG L, et al. Astragaloside IV reduces the hypoxia-induced injury in PC-12 cells by inhibiting expression of miR-124[J]. *Biomed Pharmacother*, 2018, 106:419-425.
- [27] GONG L C, CHANG H, ZHANG J Z, et al. Astragaloside IV protects rat cardiomyocytes from hypoxia-induced injury by down-regulation of miR-23a and miR-92a[J]. *Cell Physiol Biochem*, 2018, 49(6):2240-2253. (收稿日期:2020-11-29)