

研究论文

水苏碱调节EPAC1/Rap1信号通路对H/R诱导的
心肌细胞凋亡的影响

刘盼* 赵媛媛 杜长洪

(广元市第一人民医院心血管内科, 广元 628000)

摘要 该文旨在探究水苏碱(STA)调节环腺苷酸直接激活的交换蛋白1(EPAC1)/Ras相关蛋白1(Rap1)信号通路对缺氧/复氧(H/R)诱导的心肌细胞凋亡的影响。将H9C2细胞分为对照组、模型组(H/R)、H/R+STA(5 $\mu\text{mol/L}$ STA)组、H/R+8-CPT(EPAC1激动剂8-CPT, 30 $\mu\text{mol/L}$)组、H/R+STA+8-CPT组(5 $\mu\text{mol/L}$ STA+30 $\mu\text{mol/L}$ 8-CPT)、H/R+ESI-09(EPAC1抑制剂ESI-09, 1 $\mu\text{mol/L}$)组、H/R+STA+ESI-09组(5 $\mu\text{mol/L}$ STA+1 $\mu\text{mol/L}$ ESI-09)。利用CCK-8法检测H9C2细胞增殖情况;DCFH-DA法检测H9C2细胞活性氧(ROS)水平;流式细胞术检测H9C2细胞凋亡情况;实时荧光定量PCR(qRT-PCR)检测心肌细胞中EPAC1、Rap1的mRNA表达水平;Western blot检测心肌细胞中EPAC1、Rap1、Bcl-2相关X蛋白(Bax)、B淋巴细胞瘤-2(Bcl-2)蛋白表达水平。结果显示:与对照组比较,H/R组H9C2细胞中ROS水平、细胞凋亡率、Bax蛋白表达水平以及EPAC1、Rap1的mRNA及蛋白表达水平均显著升高,细胞增殖活力和Bcl-2蛋白表达水平显著降低($P<0.05$);与H/R组相比,H/R+STA组和H/R+ESI-09组H9C2细胞中ROS水平、细胞凋亡率、Bax蛋白表达水平以及EPAC1、Rap1的mRNA及蛋白表达水平均显著降低,细胞增殖活力和Bcl-2蛋白表达水平显著升高($P<0.05$),而H/R+8-CPT组指标与上述趋势相反($P<0.05$);与H/R+STA组相比,H/R+STA+8-CPT组H9C2细胞中ROS水平、细胞凋亡率、Bax蛋白表达水平以及EPAC1、Rap1的mRNA及蛋白表达水平均显著升高,细胞增殖活力和Bcl-2蛋白表达水平显著降低($P<0.05$),而H/R+STA+ESI-09组指标与上述趋势相反($P<0.05$)。总之,STA可能通过抑制EPAC1/Rap1信号通路,改善由H/R诱导的心肌细胞凋亡。

关键词 水苏碱;环磷酸腺苷直接激活的交换蛋白1;Ras相关蛋白1;缺氧/复氧;心肌细胞;凋亡

The Impacts of Stachydrine on H/R Induced Apoptosis of Myocardial Cells
by Regulating the EPAC1/Rap1 Signaling Pathway

LIU Pan*, ZHAO Yuanyuan, DU Changhong

(Department of Cardiovascular Medicine, Guangyuan First People's Hospital, Guangyuan 628000, China)

Abstract The aim of this study was to investigate the impacts of STA (stachydrine) on H/R (hypoxia/reoxygenation) induced apoptosis of myocardial cells by regulating the EPAC1 (exchange protein 1 directly activated

收稿日期: 2023-08-17 接受日期: 2023-10-19

2019年四川省第一批科技计划项目(批准号: 2019YJ0702)资助的课题

*通讯作者。Tel: 15984448300, E-mail: liupan11@163.com

Received: August 17, 2023

Accepted: October 19, 2023

This work was supported by the First Batch of Science and Technology Plan Project of Sichuan Province in 2019 (Grant No.2019YJ0702)

*Corresponding author. Tel: +86-15984448300, E-mail: liupan11@163.com

by cAMP)/Rap1 (Ras associated protein 1) signaling pathway. H9C2 cells were grouped into control group, model group (H/R), H/R+STA (5 $\mu\text{mol/L}$ STA) group, H/R+8-CPT (EPAC1 agonist 8-CPT, 30 $\mu\text{mol/L}$) group, H/R+STA+8-CPT group (5 $\mu\text{mol/L}$ STA+30 $\mu\text{mol/L}$ 8-CPT), H/R+ESI-09 (EPAC1 inhibitor ESI-09, 1 $\mu\text{mol/L}$) group, and H/R+STA+ESI-09 (5 $\mu\text{mol/L}$ STA+1 $\mu\text{mol/L}$ ESI-09) group; CCK-8 method was applied to detect the proliferation of H9C2 cells; DCFH-DA method was applied to detect the level of ROS (reactive oxygen species) in H9C2 cells; flow cytometry was applied to detect H9C2 cell apoptosis; qRT-PCR (real-time fluorescence quantitative PCR) was applied to detect the mRNA expression levels of *EPAC1* and *Rap1* in myocardial cells; Western blot was applied to detect the expression levels of EPAC1, Rap1, Bax (Bcl-2 associated X protein), and Bcl-2 (B cell lymphocyte tumor 2) proteins in myocardial cells. Compared with the control group, the level of ROS, apoptosis rate, the protein expression of Bax, as well as the mRNA and protein expression of EPAC1 and Rap1 in H9C2 cells in the H/R group were obviously increased, while the cell proliferation activity and the protein expression of Bcl-2 were obviously reduced ($P<0.05$); compared with the H/R group, the level of ROS, apoptosis rate, the protein expression of Bax, as well as the mRNA and protein expression of EPAC1 and Rap1 in H9C2 cells in the H/R+STA group and H/R+ESI-09 group were obviously reduced, while the cell proliferation activity and the protein expression of Bcl-2 were obviously increased ($P<0.05$); the indicators in the H/R+8-CPT group showed opposite trends ($P<0.05$); compared with the H/R+STA group, the level of ROS, apoptosis rate, the protein expression of Bax, as well as the mRNA and protein expression of EPAC1 and Rap1 in H9C2 cells in the H/R+STA+8-CPT group were obviously increased, the cell proliferation activity and the protein expression of Bcl-2 were obviously reduced ($P<0.05$), the indicators in the H/R+STA+ESI-09 group showed opposite trends ($P<0.05$). In conclusion, STA may improve H/R-induced cardiomyocardial cells apoptosis by inhibiting the EPAC1/Rap1 signaling pathway.

Keywords stachydrine; exchange protein 1 directly activated by cAMP; Ras associated protein 1; hypoxia/re-oxygenation; myocardial cells; apoptosis

心肌梗死是全球冠心病患者死亡的主要原因之一, 心肌缺血再灌注是其有效治疗策略, 但该策略会导致心肌缺血再灌注损伤(myocardial ischemia/reperfusion injury, MIRI)的发生, 即血流恢复对缺血心肌造成的严重损伤^[1]。研究发现, MIRI的病理生理机制与氧化应激、细胞内钙超载、细胞凋亡等有关^[2-3], 然而其具体机制目前仍尚未被完全阐明, 因此, 探究MIRI的发生机制及寻找抑制心肌细胞凋亡的方法对于改善MIRI具有重要的意义。cAMP直接激活的交换蛋白1(exchange protein 1 directly activated by cAMP, EPAC1)是一类新的cAMP下游效应分子, 是能与cAMP直接结合的鸟苷酸转换因子(guanine nucleotide exchange factor, GEF)^[4]。有证据表明, 缺血/再灌注(ischemia/reperfusion, I/R)通过刺激cAMP的产生, 激活EPAC1, 导致线粒体钙超载、ATP产生减少和活性氧(reactive oxygen species, ROS)产生过量以及线粒体膜通透性增加, 从而促进心肌细胞凋亡^[5]。EPAC1作为Ras相关蛋白1(Ras-associated protein, Rap1)特异性GEF, 可通过与cAMP直接结合被

激活, 从而激活Rap1并通过Rap1信号通路途径发挥许多生物学作用^[6-7]。水苏碱(stachydrine, STA)存在于多种中药中, 主要集中在唇形科、菊科等^[8], 水苏碱作为益母草活性成分主要的一部分, 在心脏、肾脏等多脏器部位具有多种重要的药理活性^[9]。郭书凯等^[10]报道, STA可降低血清心肌酶含量和改善心肌细胞的能量代谢, 且具有明显的抗心肌缺血作用。但目前关于STA能否抵抗MIRI诱导的心肌细胞凋亡及能否通过下调EPAC1/Rap1信号通路来抑制MIRI的报道很少。本研究选择原代大鼠心肌细胞H9C2体外培养, 通过缺氧/复氧(hypoxia/reoxygenation, H/R)处理来模拟MIRI体外模型, 探究STA调控EPAC1/Rap1信号通路对H/R诱导的心肌细胞凋亡的影响。

1 材料与方法

1.1 材料与试剂

大鼠心肌细胞H9C2购自中国科学院上海细胞库; 盐酸水苏碱购自上海广锐生物科技有限公司;

DMEM培养基购自合肥博美生物科技有限公司; 10%胎牛血清(fetal bovine serum, FBS)购自澳大利亚CellMax公司; 青霉素-链霉素溶液、ROS检测试剂盒购自上海碧云天生物技术有限公司; 反转录试剂盒购自北京康瑞纳生物科技有限公司; BCA蛋白定量试剂盒购自南京凯基生物技术有限公司; 实时荧光定量PCR(quantitative Real-time PCR, qRT-PCR)试剂盒购自广州英赞生物技术有限公司; Trizol试剂购自美国LIFE公司; MTT检测试剂购自美国Sigma公司; EPCA1激动剂(8-CPT)、兔源一抗Bcl-2相关X蛋白(Bcl-2-associated X protein, Bax)均购自艾美捷科技有限公司; EPAC1抑制剂(ESI-09)购自上海源叶生物科技有限公司; 兔源一抗EPCA1、Rap1、GAPDH购自北京索莱宝科技有限公司; 兔源一抗B淋巴细胞瘤-2(B-cell lymphoma-2, Bcl-2)购自北京普利莱基因技术有限公司; 辣根过氧化物酶(horse-radish peroxidase, HRP)标记的羊抗兔二抗购自英国Abcam公司。

1.2 细胞培养

在含有10% FBS和1%青霉素-链霉素的DMEM培养基中培养H9C2细胞, 培养条件为37 °C、5% CO₂。

1.3 细胞培养与分组

H/R细胞模型的构建^[11]: 在缺氧的条件下(95% N₂和5% CO₂), 将H9C2细胞置于无FBS的DMEM培养基中培养6 h, 诱导缺氧; 缺氧后, 将H9C2细胞在有氧条件(95% O₂和5% CO₂)下, 于含有10% FBS的DMEM培养基中培养12 h, 以构建H/R细胞模型。将在正常条件下, 含有95% O₂和5% CO₂的培养箱中培养的H9C2细胞作为对照组。

将H9C2细胞随机分为以下几组: 对照组、模型组(H/R组)、H/R+STA(5 μmol/L STA)^[12]组、H/R+8-CPT组(EPCA1激动剂8-CPT, 30 μmol/L)^[13]、H/R+STA+8-CPT组(5 μmol/L STA+30 μmol/L 8-CPT)、H/R+ESI-09组(EPCA1抑制剂ESI-09, 1 μmol/L)^[13]、H/R+STA+ESI-09组(5 μmol/L STA+1 μmol/L ESI-09)。对照组H9C2细胞进行正常培养, H/R组H9C2细胞进行H/R处理, 其他给药组H9C2细胞给药预处理12 h后再进行H/R处理。

1.4 CCK-8法检测H9C2细胞增殖情况

将各组的H9C2细胞以密度1×10⁴个/孔接种到96孔板中。每组设置5个复孔。当细胞密度达到约

80%时, 按照10 μL/孔加入CCK-8试剂, 在37 °C培养箱中继续孵育2 h。使用酶联免疫吸附测定法测量波长为450 nm时的吸光度(D)值。

1.5 DCFH-DA法检测H9C2细胞中ROS含量

将各组的H9C2细胞以密度5×10⁴个/孔接种到24孔板中, 每组设置5个复孔。当细胞密度达到约80%时, 弃去上清培养基, 用磷酸盐缓冲液(phosphate buffered saline, PBS)洗涤1次, 加入500 μL浓度为10 μmol/L的DCFH-DA, 放入37 °C培养箱中孵育20 min。吸去染色液, 每孔加入1 mL无血清的培养基进行洗涤, 重复3次。在激发波长为488 nm, 发射波长为525 nm的激光共聚焦显微镜下检测H9C2细胞中ROS含量并进行拍照。

1.6 流式细胞术检测H9C2细胞凋亡情况

将各组H9C2细胞以密度5×10⁴个/孔接种在24孔板中, 每组设置5个复孔。当细胞密度达到约80%时, 弃去上清培养基后加入胰蛋白酶于37 °C消化3 min, 收集细胞。将各组细胞重悬在1 mL 1×结合缓冲液(binding buffer)中, 根据相关试剂盒说明书加入5 μL Annexin V-FITC和5 μL PI, 室温避光孵育15 min, 通过流式细胞仪检测细胞凋亡情况, 测定细胞凋亡率。

1.7 qRT-PCR检测H9C2细胞EPAC1、Rap1的表达水平

将各组H9C2细胞以密度1×10⁶个/孔接种在6孔细胞培养板中, 每组设置5个复孔, 当细胞密度达到80%左右时, 弃去上清培养基, 用PBS洗涤1次, 收集各组细胞, 用Trizol试剂提取细胞总RNA, 测定RNA浓度和纯度。使用反转录试剂盒反转成cDNA。以cDNA为模板进行qRT-PCR反应。反应体系为20 μL, 扩增条件: 95 °C预变性5 min; 95 °C变性30 s, 60 °C退火10 s, 72 °C延伸2 s, 循环40次。引物序列设计如表1所示。以GAPDH为内参, 使用2^{-ΔΔCt}公式计算各组中EPAC1、Rap1的mRNA表达水平。

1.8 Western blot检测H9C2细胞中EPAC1/Rap1信号通路及凋亡相关蛋白表达水平

收集各组H9C2细胞。每孔加入100 μL RIPA裂解缓冲液, 提取各组细胞中的总蛋白。用BCA蛋白定量试剂盒进行总蛋白定量。上样后进行SDS-PAGE凝胶电泳, 电泳结束后用半干转法转移至PVDF膜上, 脱脂奶粉室温封闭1 h, 将PVDF膜与一抗EPAC1(稀释比例为1:1 000)、Rap1(稀释比例为

表1 引物设计序列

Table 1 Primers design sequence

基因	引物序列
Gene	Primer sequence
EPAC1	Forward: 5'-GCA CGC TGC TCA ATA TGG TG-3'
	Reverse: 5'-CGG TGC TCG AAC ACT AGC TG-3'
Rap1	Forward: 5'-ATG CGT GAG TAC AAG CTA GTA GT-3'
	Reverse: 5'-AAT CTA CCT CGA CTT GCT TTC TG-3'
GAPDH	Forward: 5'-ACA GCA ACA GGG TGG TGG AC-3'
	Reverse: 5'-TTT GAG GGT GCA GCG AAC TTT-3'

1:1 000)、Bcl-2(稀释比例为1:1 000)、GAPDH(稀释比例为1:1 000)、Bax(稀释比例为1:1 000)在4 ℃下孵育过夜,用1× TBST洗膜,5 min/次,共3次;再加入HRP标记的二抗(稀释比例为1:3 000)在室温下孵育4 h。加入ECL试剂观察蛋白显色情况,通过Quantity One软件评估蛋白灰度值。

1.9 统计学分析

使用GraphPad Prism 6软件进行统计学分析,计量资料表示为平均值±标准差($\bar{x}\pm s$)。单因素方差分析用于多组间的比较,进一步两组间的比较采用SNK-*q*检验, $P<0.05$ 为差异具有显著性。

2 结果

2.1 STA对H9C2细胞增殖的影响

根据图1可知:与对照组相比,H/R组H9C2细胞增殖活力显著降低($P<0.05$);与H/R组相比,H/R+STA组、H/R+ESI-09组H9C2细胞增殖活力显著升高

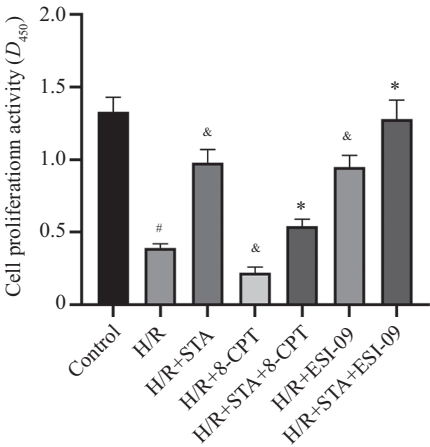
($P<0.05$),H/R+8-CPT组H9C2细胞增殖活力显著降低($P<0.05$);与H/R+STA组相比,H/R+STA+8-CPT组H9C2细胞增殖活力显著降低,H/R+STA+ESI-09组H9C2细胞增殖活力显著升高($P<0.05$)。

2.2 STA对H9C2细胞中ROS含量的影响

根据图2可知:与对照组相比,H/R组H9C2细胞中ROS水平显著升高($P<0.05$);与H/R组相比,H/R+STA组、H/R+ESI-09组H9C2细胞中ROS水平显著降低($P<0.05$),H/R+8-CPT组H9C2细胞中ROS水平显著升高($P<0.05$);与H/R+STA组相比,H/R+STA+8-CPT组H9C2细胞中ROS水平显著升高,H/R+STA+ESI-09组H9C2细胞中ROS水平显著降低($P<0.05$)。

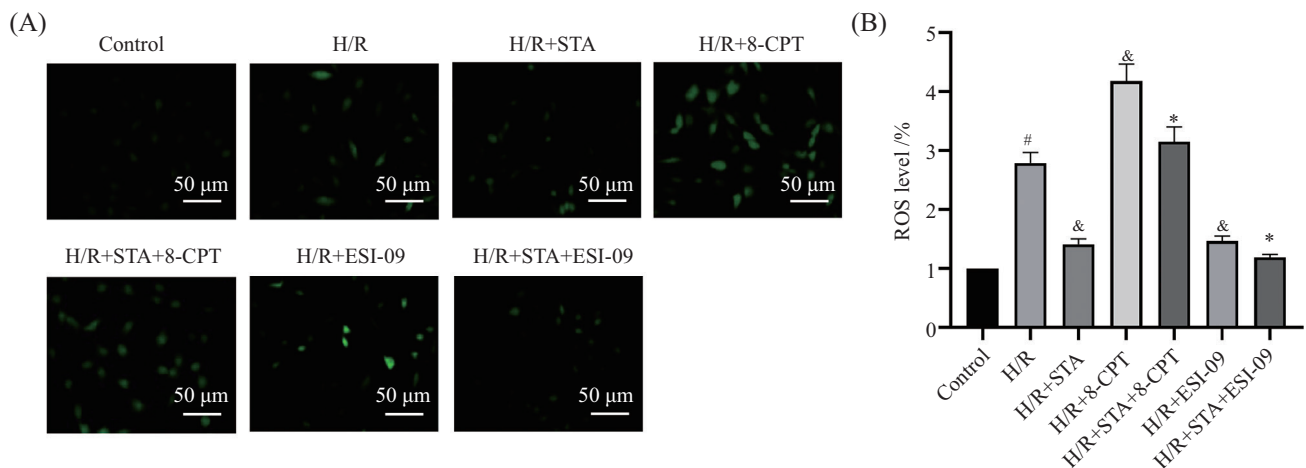
2.3 STA对H9C2细胞凋亡的影响

根据图3可知:与对照组相比,H/R组H9C2细胞的细胞凋亡率升高($P<0.05$);与H/R组相比,H/R+STA组、H/R+ESI-09组H9C2细胞凋亡率降低($P<0.05$),H/R+8-CPT组H9C2细胞的细胞凋亡



$P<0.05$, 与对照组相比; & $P<0.05$, 与H/R组相比; * $P<0.05$, 与H/R+STA组相比。 $n=5$ 。
$P<0.05$ compared with Control group; & $P<0.05$ compared with H/R group; * $P<0.05$ compared with H/R+STA group. $n=5$.

图1 各组H9C2细胞增殖活力比较
Fig.1 Comparison of H9C2 cell proliferation activity in each group

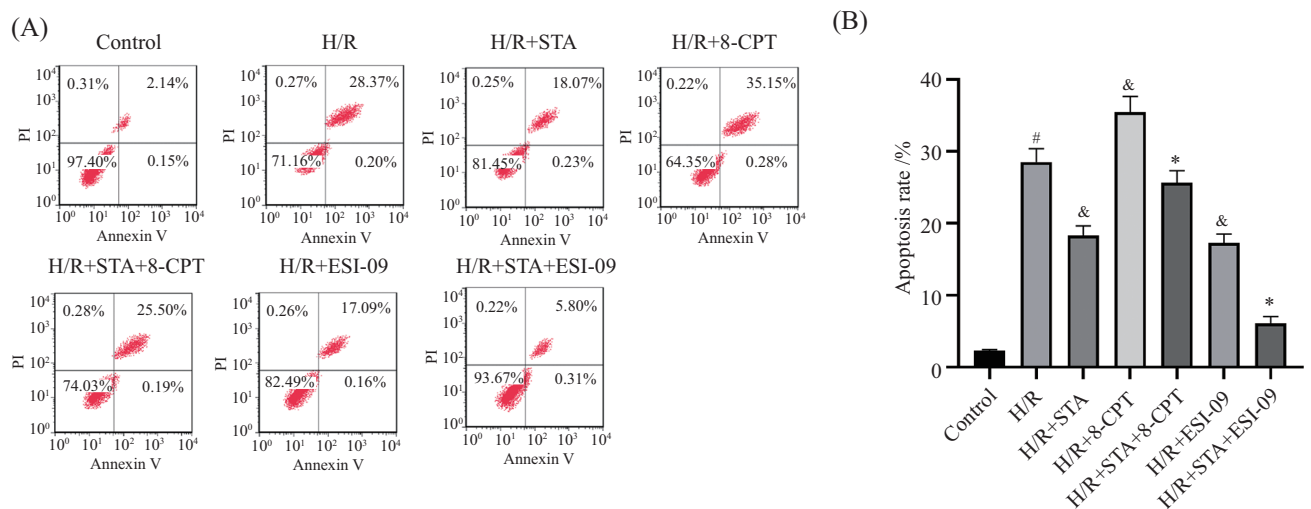


A: DCFH-DA法检测H9C2细胞中的ROS水平; B: 各组H9C2细胞中ROS水平比较。* $P<0.05$, 与对照组相比; & $P<0.05$, 与H/R组相比; * $P<0.05$, 与H/R+STA组相比。n=5。

A: the ROS level in H9C2 cells was detected by DCFH-DA method; B: comparison of ROS levels in H9C2 cells in each group. * $P<0.05$ compared with Control group; & $P<0.05$ compared with H/R group; * $P<0.05$ compared with H/R+STA group. n=5.

图2 各组H9C2细胞中ROS水平变化

Fig.2 Changes of ROS levels in H9C2 cells in each group



A: 流式细胞术检测H9C2细胞凋亡; B: 各组H9C2细胞凋亡率比较。* $P<0.05$, 与对照组相比; & $P<0.05$, 与H/R组相比; * $P<0.05$, 与H/R+STA组相比。n=5。

A: the apoptosis of H9C2 cells was detected by flow cytometry; B: comparison of apoptosis rate of H9C2 cells in each group. * $P<0.05$ compared with Control group; & $P<0.05$ compared with H/R group; * $P<0.05$ compared with H/R+STA group. n=5.

图3 各组H9C2细胞凋亡情况

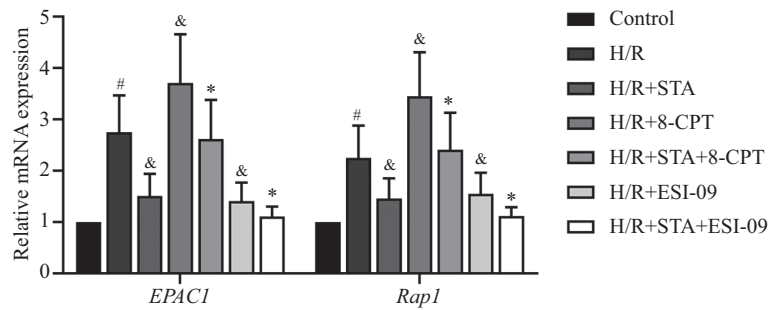
Fig.3 Apoptosis of H9C2 cells in each group

率升高($P<0.05$); 与H/R+STA组相比, H/R+STA+8-CPT组凋亡率升高, H/R+STA+ESI-09组H9C2细胞凋亡率降低($P<0.05$)。

2.4 STA对H9C2细胞EPAC1、Rap1的mRNA表达水平的影响

根据图4可知: 与对照组相比, H/R组H9C2细胞EPAC1、Rap1的mRNA表达水平均明显升高

($P<0.05$); 与H/R组相比, H/R+STA组、H/R+ESI-09组H9C2细胞中EPAC1和Rap1的mRNA表达水平明显降低($P<0.05$), H/R+8-CPT组H9C2细胞中EPAC1和Rap1的mRNA表达水平明显升高($P<0.05$); 与H/R+STA组相比, H/R+STA+8-CPT组EPAC1和Rap1的mRNA表达水平明显升高, H/R+STA+ESI-09组H9C2细胞中EPAC1和Rap1的mRNA表达水平明显降低

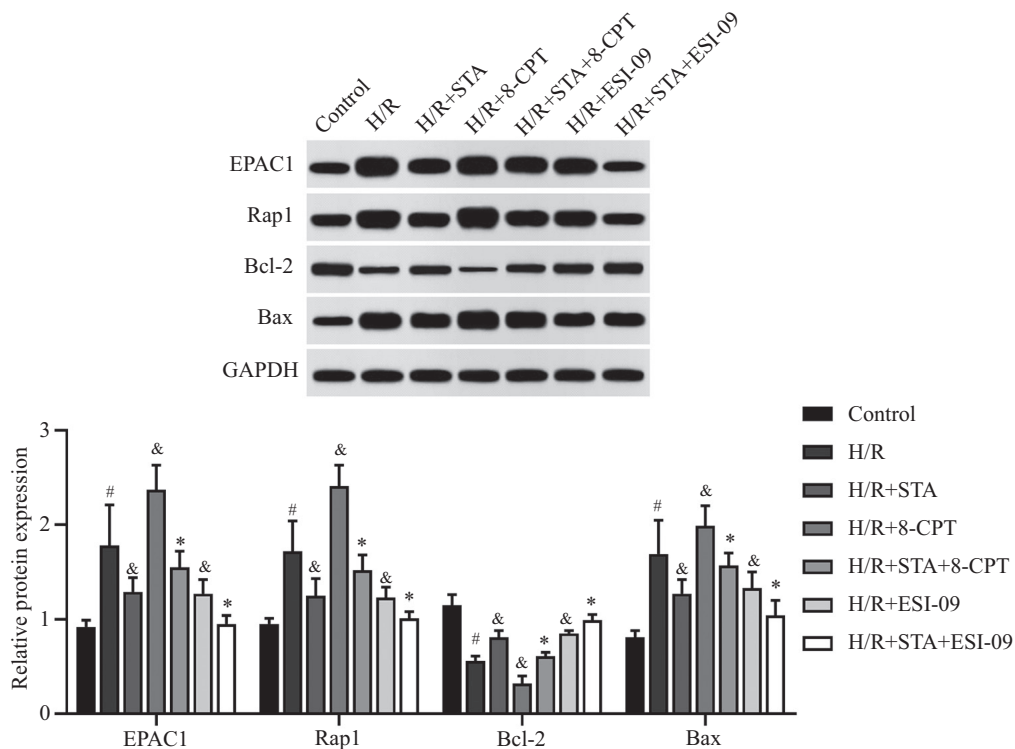


[#] $P<0.05$, 与对照组相比; [&] $P<0.05$, 与H/R组相比; ^{*} $P<0.05$, 与H/R+STA组相比。 $n=5$ 。

[#] $P<0.05$ compared with Control group; [&] $P<0.05$ compared with H/R group; ^{*} $P<0.05$ compared with H/R+STA group. $n=5$ 。

图4 各组H9C2细胞EPAC1和Rap1的mRNA水平比较

Fig.4 Comparison of mRNA levels of EPAC1 and Rap1 in H9C2 cells in each group



[#] $P<0.05$, 与对照组相比; [&] $P<0.05$, 与H/R组相比; ^{*} $P<0.05$, 与H/R+STA组相比。 $n=5$ 。

[#] $P<0.05$ compared with Control group; [&] $P<0.05$ compared with H/R group; ^{*} $P<0.05$ compared with H/R+STA group. $n=5$ 。

图5 各组H9C2细胞中的EPAC1、Rap1、Bcl-2、Bax蛋白水平的比较

Fig.5 Comparison of EPAC1, Rap1, Bcl-2 and Bax protein levels in H9C2 cells in each group

($P<0.05$)。

2.5 STA对H9C2细胞中EPAC1/Rap1信号通路及凋亡相关蛋白表达的影响

根据图5可知: 与对照组相比, H/R组的H9C2细胞中EPAC1、Rap1和Bax的蛋白表达量明显增加, Bcl-2蛋白表达量明显减少($P<0.05$); 与H/R组相比, H/R+STA组和H/R+ESI-09组H9C2细胞中EPAC1、Rap1和Bax蛋白表达量明显减少, Bcl-2蛋白表达

量明显增加($P<0.05$), H/R+8-CPT组H9C2细胞中EPAC1、Rap1和Bax蛋白表达量增加, Bcl-2蛋白表达量明显减少($P<0.05$); 与H/R+STA组相比, H/R+STA+8-CPT组H9C2细胞中EPAC1、Rap1和Bax蛋白表达量明显增加, Bcl-2蛋白表达量明显减少, H/R+STA+ESI-09组H9C2细胞中EPAC1、Rap1和Bax蛋白的表达量明显减少, Bcl-2蛋白表达量明显增加($P<0.05$)。

3 讨论

MIRI可导致缺血心肌损伤加重,过去研究发现,心肌再灌注可导致更多的心肌细胞死亡,从而进一步使患者心功能恶化,导致治疗效果不佳^[14]。在临床缺血性心脏病治疗中,由于MIRI涉及的机制较多,比如氧化应激、细胞凋亡等,所以MIRI在治疗方面是一大难题^[15]。由此,需要寻找一种新的有效治疗药物以改善MIRI。

STA是益母草的主要生物成分,已被证明具有广泛的生物活性,包括抗炎、抗氧化、抗细胞凋亡等^[16]。刘红燕等^[17]研究表明,在过氧化氢所致肾小管上皮细胞损伤中,低剂量STA可通过上调Bcl-2蛋白表达减轻肾小管上皮细胞损伤并减少细胞凋亡。STA能有效改善异丙肾上腺素诱发的心肌纤维化^[18]。本研究发现H/R诱导心肌损伤后,ROS水平、细胞凋亡率、Bax表达水平均升高,细胞增殖活性、Bcl-2蛋白水平降低;经过STA预处理后,细胞增殖活性、Bcl-2蛋白水平升高,细胞凋亡率、Bax表达水平和ROS水平均降低。这提示STA对H/R损伤细胞具有修复作用,能够抑制细胞凋亡,降低ROS水平。

EPAC1是一种大小约为100 kDa的单体蛋白。近年来对其在中枢和外周疾病中的潜在作用进行了大量研究,EPAC1主要存在于心脏、中枢神经系统等中,参与细胞增殖和细胞凋亡,并作用于下游效应分子Rap1^[6-7,19-20]。FAZAL等^[21]的研究表明,通过敲除EPAC1基因可减少心肌梗死面积并抑制细胞凋亡,从而阻滞实验性MIRI的发生。因此,本研究用EPAC1激动剂8-CPT进行预处理,实验结果显示:与H/R组相比,给予EPAC1激动剂8-CPT预处理后心肌细胞损伤进一步加重,而给予EPAC1抑制剂ESI-09预处理后H/R诱导的心肌细胞损伤有所缓解;且STA+ESI-09预处理可以进一步缓解H/R诱导的心肌损伤,显著降低细胞凋亡率、ROS水平以及EPAC1、Rap1和Bax的表达水平,增加细胞增殖活性、Bcl-2蛋白表达量,提示EPAC1/Rap1信号途径可能参与H/R诱导的心肌细胞损伤过程,且STA减轻H/R诱导的心肌细胞损伤,可能是通过抑制EPAC1/Rap1信号途径来实现的,EPAC1/Rap1信号通路有望作为治疗MIRI的有效靶点。

综上所述,STA可通过抑制EPAC1/Rap1信号途径减轻H/R诱导的心肌细胞损伤。本研究不仅对MIRI的研究具有重要意义,还对寻找新型治疗MIRI

的药物有一定参考价值。后续,本研究也将继续通过RNA干扰技术或基因编辑技术更加精确地干扰EPAC1/Rap1信号通路,进一步探究EPAC1/Rap1信号通路在水苏碱改善由H/R诱导的心肌细胞凋亡中发挥的作用,并通过体内实验进行探索验证。

参考文献 (References)

- [1] HE J, LIU D, ZHAO L, et al. Myocardial ischemia/reperfusion injury: mechanisms of injury and implications for management [J]. *Exp Ther Med*, 2022, 23(6): 430.
- [2] LIU Y, ZHANG J, ZHANG D, et al. Research progress on the role of pyroptosis in myocardial ischemia-reperfusion injury [J]. *Cells*, 2022, 11(20): 3271.
- [3] CAO Y, LUO F, PENG J, et al. KMT2B-dependent RfK transcription activates the TNF- α /NOX2 pathway and enhances ferroptosis caused by myocardial ischemia-reperfusion [J]. *Mol Cell Cardiol*, 2022, 173(1): 75-91.
- [4] JIANG Y, STEINLE J J. Epac1 requires AMPK phosphorylation to regulate HMGB1 in the retinal vasculature [J]. *Invest Ophthalmol Vis Sci*, 2020, 61(11): 331-5.
- [5] LIU L, JIANG Y, STEINLE J J. Epac1 and PKA agonists inhibit ROS to reduce NLRP3 inflammasome proteins in retinal endothelial cells [J]. *Mol Vis*, 2022, 28(1): 500-6.
- [6] CHUAN W, YUAN L, WEN J, et al. cAMP-Epac1 signaling is activated in DDAVP-induced endolymphatic hydrops of guinea pigs [J]. *Braz J Otorhinolaryngol*, 2023, 89(3): 469-76.
- [7] WANG C, LI Y, LIU W, et al. The expression and significance of Epac1 and Epac2 in the inner ear of guinea pigs [J]. *Eur Arch Otorhinolaryngol*, 2022, 279(11): 5207-14.
- [8] LIAO L, TANG Y, LI B, et al. Stachydrine, a potential drug for the treatment of cardiovascular system and central nervous system diseases [J]. *Biomed Pharmacother*, 2023, 161(1): 1144891-7.
- [9] GU R, ZHANG W, XU D. Stachydrine is effective and selective against blast phase chronic myeloid leukaemia through inhibition of multiple receptor tyrosine kinases [J]. *Pharm Biol*, 2022, 60(1): 700-7.
- [10] 郭书凯, 周艳丽, 陈伟, 等. 水苏碱对急性心肌梗死小鼠的保护作用[J]. 泰山医学院学报(GUO S K, ZHOU Y L, CHEN W, et al. Protective effect of hydrostachyline on acute myocardial ischemia in mice [J]. *Journal of Taishan Medical College*), 2018, 39(8): 844-6.
- [11] HUANG J, QI Z. MiR-21 mediates the protection of kaempferol against hypoxia/reoxygenation-induced cardiomyocyte injury via promoting Notch1/PTEN/AKT signaling pathway [J]. *PLoS One*, 2020, 15(11): e0241007.
- [12] 曲良超, 黄佳佳, 范明达, 等. 水苏碱对阿尔茨海默病体外模型A β -(25-35)诱导PC12细胞凋亡的影响[J]. 南方医科大学学报(QU L C, HUANG J J, FAN M D, et al. Effect of hydrostachyline on apoptosis of PC12 cells induced by A β -(25-35) *in vitro* model of Alzheimer's disease [J]. *Journal of Southern Medical University*), 2020, 40(7): 1023-8.
- [13] YANG H, XUE W, DING C, et al. Vitexin mitigates myocardial ischemia/reperfusion injury in rats by regulating mitochondrial dysfunction via Epac1-Rap1 signaling [J]. *Oxid Med Cell Lon-*

- gev, 2021, 2021(1): 1-17.
- [14] HOU M, WU X, ZHAO Z, et al. Endothelial cell-targeting, ROS-ultrasensitive drug/siRNA co-delivery nanocomplexes mitigate early-stage neutrophil recruitment for the anti-inflammatory treatment of myocardial ischemia reperfusion injury [J]. *Acta Biomater*, 2022, 143(1): 344-55.
- [15] KORSHUNOVA A Y, BLAGONRAVOV M L, NEBORAK E V, et al. BCL2-regulated apoptotic process in myocardial ischemia reperfusion injury [J]. *Int J Mol Med*, 2021, 47(1): 23-36.
- [16] LI X Q, LU S, XIA L, et al. Stachydrine hydrochloride ameliorates cardiac hypertrophy through CaMKII/HDAC4/MEF2C signal pathway [J]. *Am J Transl Res*, 2022, 14(6): 3840-53.
- [17] 刘红燕, 王瑞, 石明, 等. 小剂量盐酸水苏碱对过氧化氢所致肾小管上皮细胞凋亡的保护作用[J]. *中国中西医结合肾病杂志*(LIU H Y, WANG R, SHI M, et al. Protective effect of hydrostachyline hydrochloride on apoptosis of renal tubular epithelial cells induced by hydrogen peroxide [J]. *Chinese Journal of Integrative Nephropathy*), 2008, 1(9): 760-3.
- [18] 刘胜男, 杨丹, 马宵雁, 等. 水苏碱对异丙肾上腺素致小鼠心肌纤维化的保护作用[J]. *泰山医学院学报*(LIU S N, YANG D, MA X Y, et al. Protective effect of hydrostachyline on myocardial fibrosis induced by isoproterenol in mice [J]. *Journal of Taishan Medical College*), 2020, 41(9): 655-8.
- [19] YANG W X, LIU Y, ZHANG S M, et al. Epac activation ameliorates tubulointerstitial inflammation in diabetic nephropathy [J]. *Acta Pharmacol Sin*, 2022, 43(3): 659-71.
- [20] HERNÁNDEZ C, GÓMEZ-PERALTA F, SIMÓ-SERVAT O, et al. Usefulness of circulating EPAC1 as biomarkers of therapeutic response to GLP-1 receptor agonists [J]. *Acta Diabetol*, 2022, 59(11): 1437-42.
- [21] FAZAL L, LAUDETTE M, PAULA-GOMES S, et al. Multifunctional mitochondrial Epac1 controls myocardial cell death [J]. *Circ Res*, 2017, 120(4): 645-57.