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仙茅苷调节JAK2/STAT3信号通路对红藻氨酸体外诱导的小胶质细胞损伤的影响

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摘要 该文探究仙茅苷(CCG)调节Janus蛋白酪氨酸激酶2(JAK2)/信号转导和转录激活因子3(STAT3)信号通路对红藻氨酸(KA)体外诱导的小胶质细胞损伤的影响。将HMC3细胞随机分为HMC3组、KA组、L-CCG组、M-CCG组、H-CCG组、H-CCG+JAK2/STAT3通路激活剂colivelin组, HMC3组不做干预, KA组用600 μmol/L KA刺激, L-CCG组、M-CCG组、H-CCG组在KA组基础上分别加5、10、20 μmol/L CCG, H-CCG+colivelin组在H-CCG组基础上加0.5 μmol/L colivelin。EdU法检测细胞增殖情况; Hochest 33258/PI法检测细胞凋亡情况; DCFH-DA法检测活性氧(ROS)表达情况; γ组蛋白H2A变异体(γ-H2AX)免疫荧光法检测DNA损伤情况; 试剂盒检测细胞中烟酰胺腺嘌呤二核苷磷酸/还原型烟酰胺腺嘌呤二核苷酸磷酸($NADP^+$/NADPH)、白细胞介素-1β(IL-1β)、肿瘤坏死因子-α(TNF-α)、IL-6水平; 免疫印迹法检测磷酸化JAK2(p-JAK2)、磷酸化STAT3(p-STAT3)、NOD样受体热蛋白结构域相关蛋白3(NLRP3)、含半胱氨酸的天冬氨酸蛋白水解酶1(Caspase-1)、凋亡相关斑点样蛋白(ASC)表达情况。结果显示, 与HMC3组比, KA组HMC3细胞增殖率降低, 凋亡率、γ-H2AX核焦点数量、$NADP^+$/NADPH、ROS、IL-1β、TNF-α、IL-6水平增加(P<0.05); 与KA组比, L-CCG组、M-CCG组、H-CCG组增殖率依次增加, 凋亡率、γ-H2AX核焦点数量、$NADP^+$/NADPH、ROS、IL-1β、TNF-α、IL-6水平依次降低(P<0.05); 与H-CCG组比, H-CCG+colivelin组增殖率降低, 凋亡率、γ-H2AX核焦点数量、$NADP^+$/NADPH、ROS、IL-1β、TNF-α、IL-6水平增加(P<0.05)。与HMC3组比, KA组HMC3细胞中p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达水平增加(P<0.05); 与KA组比, L-CCG组、M-CCG组、H-CCG组p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达水平依次降低(P<0.05); 与H-CCG组比, H-CCG+colivelin组p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达水平增加(P<0.05)。总之, CCG可能通过抑制JAK2/STAT3通路对KA诱导的小胶质细胞功能损伤发挥保护作用。

关键词 仙茅苷; Janus蛋白酪氨酸激酶2/信号转导和转录激活因子3通路; 红藻氨酸; 小胶质细胞功能损伤

Effect of Curculigoside on Kainic Acid Induced Microglia Injury by Regulating JAK2/STAT3 Signaling Pathway *In Vitro*

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Abstract The aim of this study was to investigate the effect of CCG (curculigoside) on microglia injury induced by KA (kainic acid) *in vitro* through regulating the JAK2 (Janus kinase-2)/STAT3 (signal transducer and activator of transcription 3) signaling pathway. HMC3 cells were randomly divided into HMC3 group, KA group, L-CCG group, M-CCG group, H-CCG group, and H-CCG+colivelin (JAK2/STAT3 pathway activator) group. The cells in the HMC3 group were not intervened, the cells in the KA group were stimulated with 600 μmol/L KA, the cells in the L-CCG, M-CCG, and H-CCG groups were added with 5, 10, and 20 μmol/L CCG, respectively, and the cells in the H-CCG+colivelin group were added with 0.5 μmol/L colivelin on the basis of the H-CCG group. The cell proliferation was detected by EdU method. Apoptosis was detected by Hochest 33258/PI method. The expression of ROS (reactive oxygen species) was detected by DCFH-DA method. DNA damage was detected by γ-H2AX (γ histone family 2A variant) immunofluorescence method. The levels of $NADP^+$/NADPH (nicotinamide adenine dinucleoside phosphate/reduced nicotinamide adenine dinucleotide phosphate), IL-1β (interleukin-1β), TNF-α (tumor necrosis factor-α), and IL-6 in cells were detected by kit. The expression of p-JAK2 (phosphorylated JAK2), p-STAT3 (phosphorylated STAT3), NLRP3 (NOD-like receptor thermal protein domain associated protein 3), Caspase-1 (cysteinyl aspartate specific proteinase 1), and ASC (apoptosis-associated speck-like protein) were detected by Western blot. The results showed that compared with the HMC3 group, the proliferation rate of HMC3 cells in the KA group was decreased, while the apoptosis rate, number of γ-H2AX nuclear foci, and the levels of $NADP^+$/NADPH, ROS, IL-1β, TNF-α, and IL-6 were increased (P<0.05). Compared with the KA group, the proliferation rate of the L-CCG group, M-CCG group, and H-CCG group were increased successively, while the apoptosis rate, number of γ-H2AX nuclear foci, and the levels of $NADP^+$/NADPH, ROS, IL-1β, TNF-α, and IL-6 were decreased successively (P<0.05). Compared with the H-CCG group, the proliferation rate of the H-CCG+colivelin group was decreased, apoptosis rate, number of γ-H2AX nuclear foci, and the levels of $NADP^+$/NADPH, ROS, IL-1β, TNF-α, and IL-6 were increased successively (P<0.05). Compared with the HMC3 group, the expression of p-JAK2, p-STAT3, NLRP3, Caspase-1, and ASC in HMC3 cells in the KA group were increased (P<0.05). Compared with the KA group, the expression of p-JAK2, p-STAT3, NLRP3, Caspase-1, and ASC in the L-CCG group, M-CCG group, and H-CCG group were decreased successively (P<0.05). Compared with the H-CCG group, the expression of p-JAK2, p-STAT3, NLRP3, Caspase-1, and ASC in the H-CCG+colivelin group were increased (P<0.05). In conclusion, CCG may have a protective effect on KA-induced microglia dysfunction by inhibiting the JAK2/STAT3 pathway.

Keywords curculigoside; Janus kinase-2/signal transducer and activator of transcription 3 pathway; kainic acid; microglia function injury

癫痫是一种以反复发作为特征的慢性脑功能障碍疾病, 在过去几十年里发病率显著增加, 需要通过药物进行治疗, 其主要药物是抗惊厥药, 可有效控制癫痫发作, 减少复发, 然而, 有约30%的患者仍存在不受控制的癫痫发作, 因此, 寻找有效的新型药物至关重要[1]。癫痫在中医上被认为是“痫病”, 痰、虚、瘀、风、惊等是其关键病因, 主要通过健脾化痰、驱邪补虚、活血化瘀、息风止痉、养心安神等方法进行治疗[2]。目前, 中药及其生物活性成分因毒性低、副作用少、易得等特点被广泛用于治疗癫痫[3]。仙茅苷(curculigoside, CCG)是提取自中药仙茅的一种天然酚类糖苷, 能通过多种通路调节细胞过程, 具有抗氧化、抗骨质疏松、抗炎、抗惊厥等药理作用[4-5]。而炎症和氧化应激通常同时出现在癫痫等神经系统疾病中, 对癫痫的发展具有重要作用[6]。近期研究显示, CCG对缺血性脑损伤、阿尔茨海默病等脑部疾病有治疗作用[7-8]。故而CCG可能通过减缓氧化应激和炎症参与治疗癫痫, 但还需进一步证实, 并探究其作用机制。Janus蛋白酪氨酸激酶2(Janus kinase-2, JAK2)是细胞因子受体的细胞内信号效应因子, 通过参与多种分子机制调控各种疾病进展[9]。信号转导和转录激活因子3(signal transducer and activator of transcription 3, STAT3)是蛋白质转录因子, 能调节免疫刺激因子表达, 与其他

通路相互作用, 参与细胞存活、分化和血管生成等过程[10]。JAK2/STAT3通路可参与活化小胶质细胞, 促进神经元凋亡, 进而损伤血脑屏障, 加重神经系统损伤, 而抑制STAT3磷酸化可减少细胞凋亡, 改善血脑屏障损伤和神经炎症, 进而保护神经元, 减轻脑组织损伤[11]。近期研究表明, 抑制JAK2/STAT3通路能够缓解癫痫症状并减轻癫痫诱导的神经损伤[12-13]。曾有报道, CCG可下调JAK2/STAT3通路, 调节凋亡蛋白表达, 参与体外骨肉瘤细胞增殖和凋亡过程[14]。但CCG能否调节JAK2/STAT3通路影响小胶质细胞还需深入探索。红藻氨酸(kainic acid, KA)可诱导小胶质细胞凋亡和氧化损伤, 被用来构建体外癫痫模型[15]。因此, 本研究从JAK2/STAT3通路出发, 以小胶质细胞为研究对象, 探究CCG对KA诱导的小胶质细胞功能损伤的影响。

1 材料与方法

1.1 材料

细胞株: 人小胶质细胞(HMC3)(货号: CRL-3304)购自美国ATCC。

药品与试剂: CCG(CAS号为85643-19-2, 分子量为466.44, 分子式为$C_{22}H_{26}O_{11}$, 纯度为HPLC≥98%)、KA(CAS号: 487-79-6)购自德国Merck公司; 胎牛血清、DMEM培养基(货号: 10100147C、11965118)购自美国Gibco公司; 细胞计数试剂盒8(cell counting kit 8, CCK8, 货号: IC-CCK8-Hu)购自上海钰博生物科技有限公司; JAK2/STAT3通路激活剂colivelin(CAS号: 867021-83-8)购自上海吉尔生化有限公司; EdU试剂盒、DNA损伤检测试剂盒、烟酰胺腺嘌呤二核苷磷酸/还原型烟酰胺腺嘌呤二核苷酸磷酸(nicotinamide adenine dinucleoside phosphate/reduced nicotinamide adenine dinucleotide phosphate, $NADP^+$/NADPH)检测试剂盒、白细胞介素-1β(interleukin-1β, IL-1β)试剂盒(货号: C0078S、C2035S、S0179、P1305)购自上海碧云天生物技术有限公司; Hochest 33258/PI凋亡试剂盒、DCFH-DA活性氧(reactive oxygen species, ROS)检测试剂盒、肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)、IL-6试剂盒(货号: YS-R987731、YSRIBIO-C0281、YS-E6101、YS-LISA3731)购自上海研生实业有限公司; 磷酸化JAK2(phosphorylated JAK2, p-JAK2)、磷酸化STAT3(phosphorylated STAT3, p-STAT3)、NOD样受体热蛋白结构域相关蛋白3(NOD-like receptor heat protein domain associated protein 3, NLRP3)、含半胱氨酸的天冬氨酸蛋白水解酶(cysteinyl aspartate specific proteinase 1, Caspase-1)、凋亡相关斑点样蛋白(apoptosis-associated speck-like protein, ASC)、甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, GAPDH)抗体(货号: 3331S、9131S、15101S、2225T、13833S、5174T)购自美国CST公司; 二抗(货号: ab97080)购自英国Abcam公司。

仪器: TH4-200倒置荧光显微镜购自日本Olympus公司, 7500型实时荧光定量PCR仪器购自美国ABI公司, Multiskan FC酶标仪购自美国ThermoFisher Scientific公司, OI 1000型凝胶成像系统购自上海山科学仪器有限公司。

1.2 方法

1.2.1 细胞培养 HMC3细胞接种至含10%胎牛血清的DMEM培养基中, 于37 °C、5% CO_2培养箱培养, 并传代。取对数期细胞用于实验。

KA诱导细胞发生氧化损伤: 向培养基中加浓度为600 μmol/L KA作用24 h, 导致胞内氧化应激和细胞损伤, 以构建体外癫痫模型[15]。

1.2.2 细胞增殖实验与分组 HMC3细胞移至96孔板, 分别用0、2.5、5、10、20、40、80 μmol/L CCG处理24 h, 加CCK-8液10 μL, 4 h后测定450 nm波长处吸光度(*D*)值。

HMC3细胞随机分为HMC3组、KA组、L-CCG组、M-CCG组、H-CCG组、H-CCG+colivelin组, HMC3组不做干预, KA组用600 μmol/L KA刺激, L-CCG组、M-CCG组、H-CCG组在KA组基础上分别加5、10、20 μmol/L CCG, H-CCG+colivelin组在H-CCG组基础上加0.5 μmol/L JAK2/STAT3通路激活剂colivelin[16]。常规培养48 h。每组3个复孔。

1.2.3 EdU法检测细胞增殖情况 HMC3细胞置于板孔, 加1 μmol/L EdU, 聚甲醛室温固定15 min, 加0.5% Triton X-100室温处理10 min, 加Click反应液室温孵育30 min, 然后用Hoechst室温孵育10 min, 荧光显微镜下观察。EdU阳性染色细胞占总细胞的百分比表示细胞增殖率。

1.2.4 Hochest 33258/PI法检测细胞凋亡情况 收集1×10^6个HMC3细胞, 用细胞染色缓冲液重悬, 分别加5 μL Hochest 33258和PI, 4 °C孵育30 min, 流式细胞仪分析凋亡率。

1.2.5 DCFH-DA法检测ROS表达情况 培养基中加1 μmol/L DCFH-DA, 37 ℃培养20 min, 洗涤细胞, 流式细胞仪使用488 nm激发波长, 525 nm发射波长分析荧光强度, 计算ROS水平。

1.2.6 组蛋白H2A变异体(γ histone family 2A variant, γ-H2AX)免疫荧光法检测DNA损伤情况 聚甲醛4 ℃固定HMC3细胞15 min, 首先加入1% Triton X-100室温封闭10 min, 加入γ-H2AX抗体(1:250) 4 ℃过夜, 再加入荧光二抗(1:500)室温反应1 h, 最后加DAPI室温孵育10 min, 用荧光显微镜观察细胞。

1.2.7 试剂盒检测细胞中$NADP^+$/NADPH 取HMC3细胞60 ℃加热30 min, 10 000 ×*g*室温离心5 min, 取适量上清移于96孔板, 加G6PDH工作液, 37 ℃避光30 min, 加显色液室温反应10 min, 测量450 nm波长处吸光度(*D*)值, 代入标准曲线计算$NADP^+$/NADPH。

1.2.8 ELISA法检测IL-1β、TNF-α、IL-6水平 HMC3细胞移至已包被抗体的板孔, 加酶标抗原37 ℃反应1 h, 加反应底物室温作用30 min, 加终止剂, 酶标仪检测特定波长处的吸光度(*D*)值, 分析IL-1β、TNF-α、IL-6水平。

1.2.9 免疫印迹法检测p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达情况 RIPA液制备细胞裂解物, BCA法测定蛋白含量, 电泳转膜, 封闭。与p-JAK2(1:1 000)、p-STAT3(1:1 000)、NLRP3(1:1 000)、Caspase-1(1:1 000)、ASC(1:1 000)、GAPDH(1:1 000)在4 ℃孵育过夜, 与二抗(1:5 000)室温孵育1.5 h, 可视化蛋白条带, 分析各蛋白表达情况。

1.3 统计学分析

采用SPSS 26.00分析数据, 数据以平均值±标准差($\bar{x}\pm s$)展示, 用单因素方差分析和SNK-*q*检验分析组间差异。$P<0.05$为差异有统计学意义。

2 结果

2.1 不同浓度CCG对HMC3存活率的影响

不同浓度CCG处理后, HMC3细胞活力增加, 5 μmol/L CCG处理24 h时, HMC3细胞活力显著增加($P<0.05$)。因此, 以20、10、5 μmol/L分别作为H-CCG组、M-CCG组、L-CCG组CCG的处理浓度(图1)。

2.2 CCG对HMC3细胞增殖率的影响

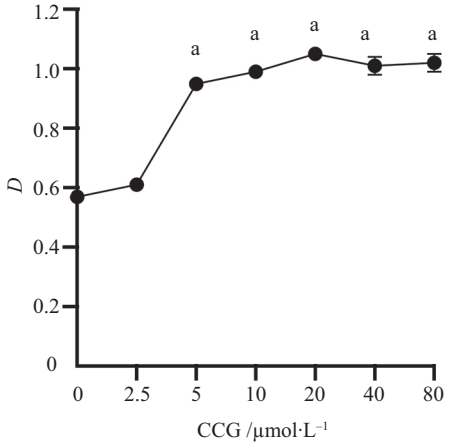
与HMC3组比, KA组HMC3细胞增殖率降低($P<0.05$); 与KA组比, L-CCG组、M-CCG组、H-CCG组增殖率依次增加($P<0.05$); 与H-CCG组比, H-CCG+colivelin组增殖率降低($P<0.05$)(图2)。

2.3 CCG对HMC3细胞凋亡率的影响

与HMC3组比, KA组HMC3细胞凋亡率增加($P<0.05$); 与KA组比, L-CCG组、M-CCG组、H-CCG组凋亡率依次降低($P<0.05$); 与H-CCG组比, H-CCG+colivelin组凋亡率增加($P<0.05$)(图3)。

2.4 CCG对HMC3细胞中ROS表达的影响

与HMC3组比, KA组HMC3细胞ROS水平增加($P<0.05$); 与KA组比, L-CCG组、M-CCG组、H-CCG组ROS水平依次降低($P<0.05$); 与H-CCG组比, H-CCG+colivelin组ROS水平增加($P<0.05$)(表1)。

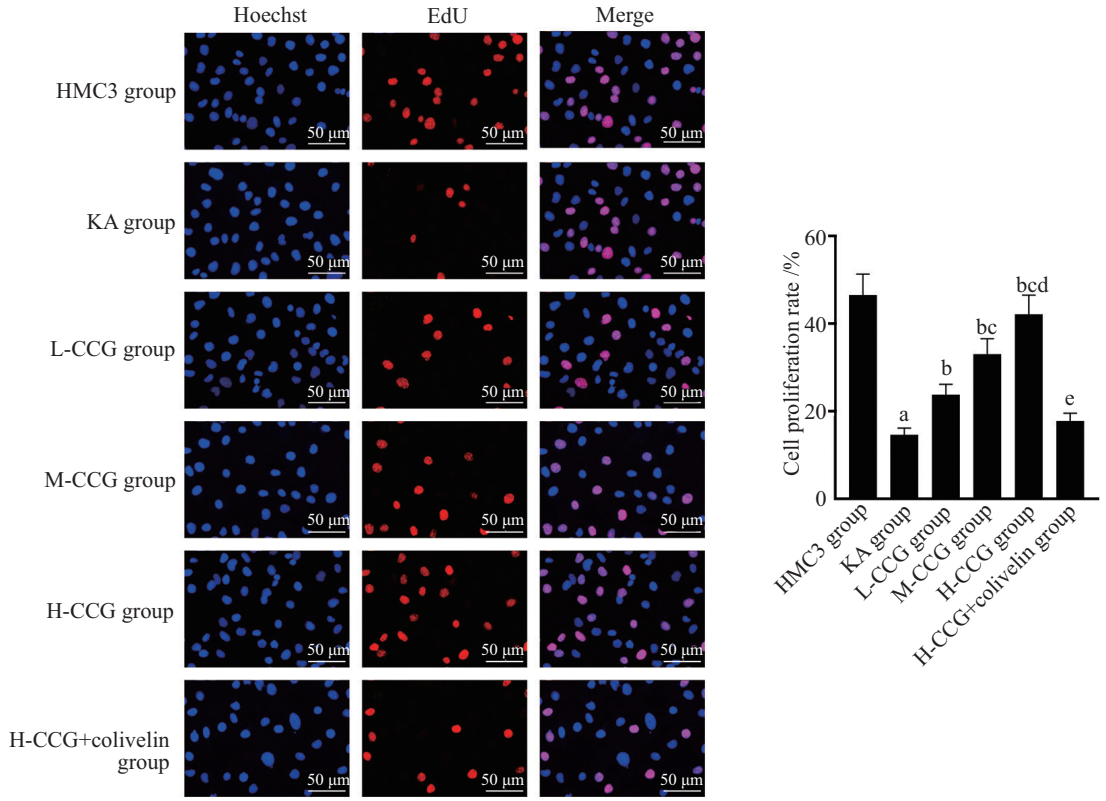


[a]$P<0.05$, 与0 μmol/L组比较。

[a]$P<0.05$ compared with 0 μmol/L group.

图1 不同浓度CCG对HMC3细胞活力的影响

Fig.1 Effect of different concentrations of CCG on the viability of HMC3 cells

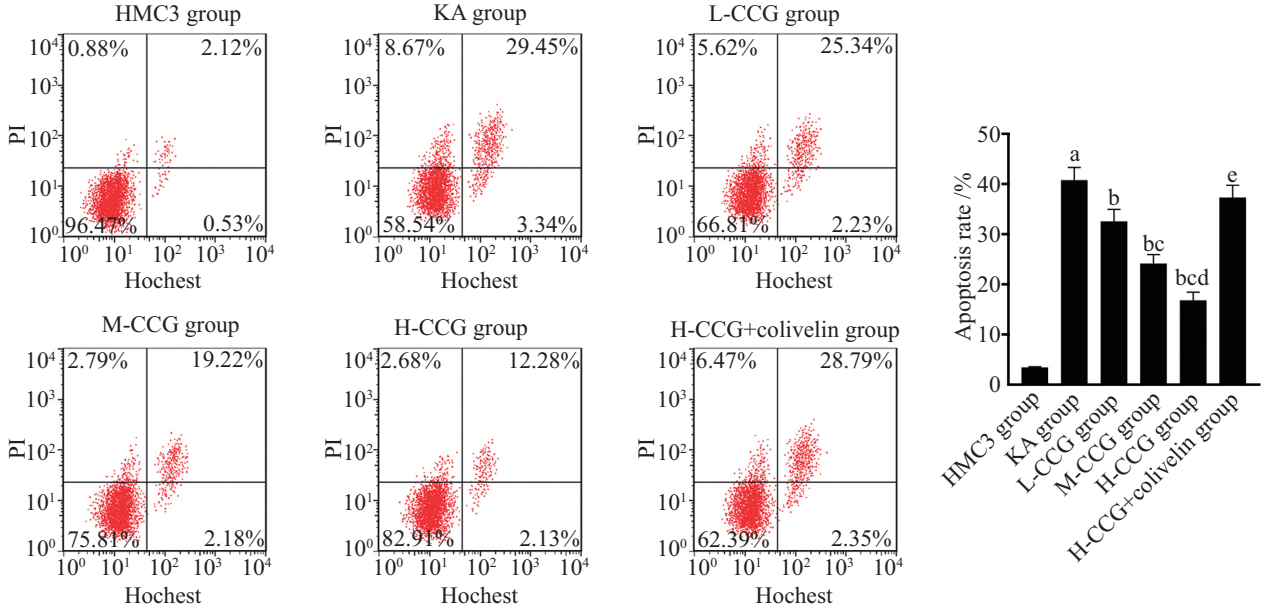


$\bar{x}\pm s$, n=6, EdU染色; [a]P<0.05, 与HMC3组比; [b]P<0.05, 与KA组比; [c]P<0.05, 与L-CCG组比; [d]P<0.05, 与M-CCG组比; [e]P<0.05, 与H-CCG组比。

$\bar{x}\pm s$, n=6, EdU staining; [a]P<0.05 compared with HMC3 group; [b]P<0.05 compared with KA group; [c]P<0.05 compared with L-CCG group; [d]P<0.05 compared with M-CCG group; [e]P<0.05 compared with H-CCG group.

图2 CCG对HMC3细胞增殖率的影响

Fig.2 Effect of CCG on HMC3 cell proliferation rate



$\bar{x}\pm s$, n=6; [a]P<0.05, 与HMC3组比; [b]P<0.05, 与KA组比; [c]P<0.05, 与L-CCG组比; [d]P<0.05, 与M-CCG组比; [e]P<0.05, 与H-CCG组比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with HMC3 group; [b]P<0.05 compared with KA group; [c]P<0.05 compared with L-CCG group; [d]P<0.05 compared with M-CCG group; [e]P<0.05 compared with H-CCG group.

图3 CCG对HMC3细胞凋亡率的影响

Fig.3 Effects of CCG on apoptosis rate of HMC3 cells

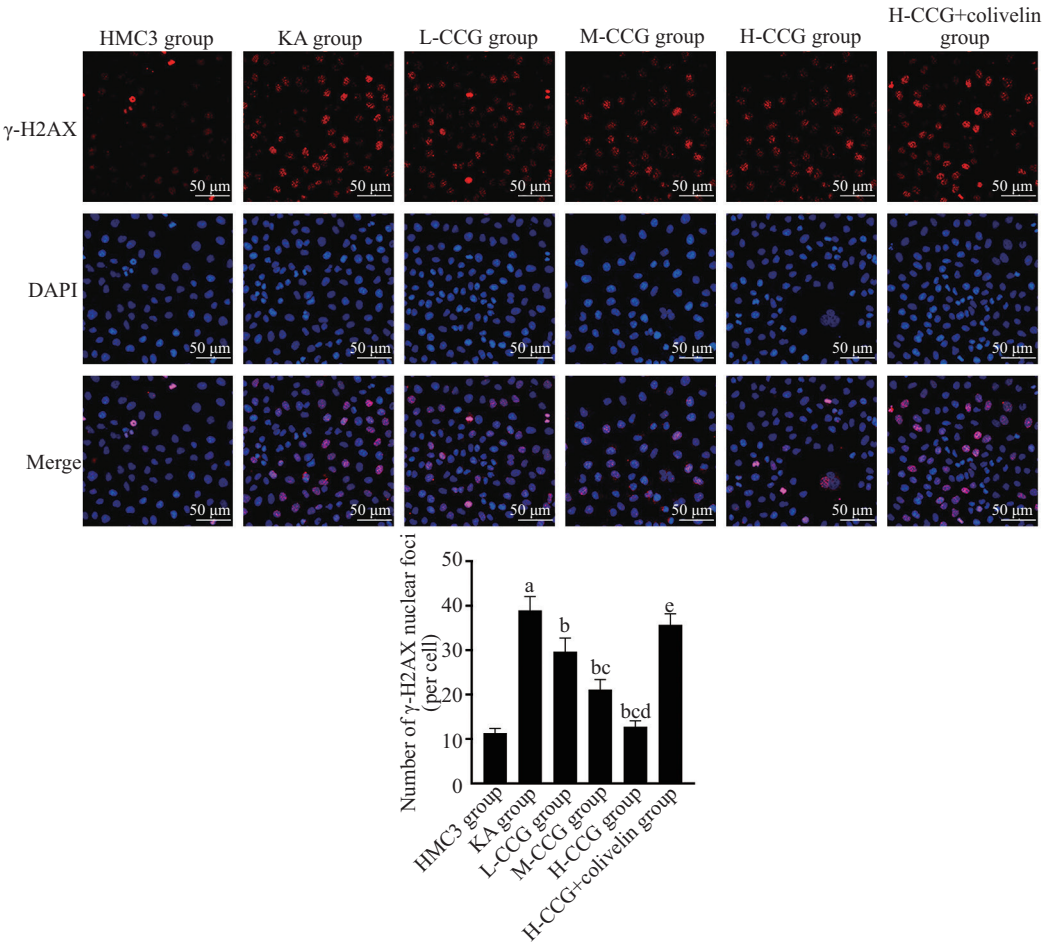
表1 CCG对HMC3细胞中ROS表达的影响

Table 1 Effects of CCG on ROS expression in HMC3 cells

组别 Group	ROS水平/% ROS level /%
HMC3 group	11.25±0.33
KA group	61.27±4.36[a]
L-CCG group	45.81±3.78[b]
M-CCG group	31.06±3.24[bc]
H-CCG group	15.43±1.15[bcd]
H-CCG+colivelin group	55.68±4.29[e]

$\bar{x}\pm s$, n=6; [a]P<0.05, 与HMC3组比; [b]P<0.05, 与KA组比; [c]P<0.05, 与L-CCG组比; [d]P<0.05, 与M-CCG组比; [e]P<0.05, 与H-CCG组比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with HMC3 group; [b]P<0.05 compared with KA group; [c]P<0.05 compared with L-CCG group; [d]P<0.05 compared with M-CCG group; [e]P<0.05 compared with H-CCG group.



$\bar{x}\pm s$, n=6, 免疫荧光染色; [a]P<0.05, 与HMC3组比; [b]P<0.05, 与KA组比; [c]P<0.05, 与L-CCG组比; [d]P<0.05, 与M-CCG组比; [e]P<0.05, 与H-CCG组比。

$\bar{x}\pm s$, n=6, immunofluorescence staining; [a]P<0.05 compared with HMC3 group; [b]P<0.05 compared with KA group; [c]P<0.05 compared with L-CCG group; [d]P<0.05 compared with M-CCG group; [e]P<0.05 compared with H-CCG group.

图4 CCG对HMC3细胞DNA损伤的影响

Fig.4 Effects of CCG on DNA damage in HMC3 cells

2.5 CCG对HMC3细胞DNA损伤的影响

与HMC3组比, KA组HMC3细胞γ-H2AX核焦点数量增加(P<0.05); 与KA组比, L-CCG组、M-CCG组、H-CCG组γ-H2AX核焦点数量依次降低(P<0.05); 与H-CCG组比, H-CCG+colivelin组γ-H2AX核焦点数量增加(P<0.05)(图4)。

2.6 CCG对NADP$^+$/NADPH的影响

与HMC3组比, KA组HMC3细胞NADP$^+$/NADPH水

表2 CCG对$NADP^+$/NADPH的影响

Table 2 Effects of CCG on $NADP^+$/NADPH

组别 Group	$NADP^+$/NADPH
HMC3 group	1.06±0.05
KA group	3.25±0.21[a]
L-CCG group	2.57±0.15[b]
M-CCG group	1.83±0.12[bc]
H-CCG group	1.12±0.14[bcd]
H-CCG+colivelin group	3.11±0.18[e]

$\bar{x}\pm s$, n=6; [a]P<0.05, 与HMC3组比; [b]P<0.05, 与KA组比; [c]P<0.05, 与L-CCG组比; [d]P<0.05, 与M-CCG组比; [e]P<0.05, 与H-CCG组比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with HMC3 group; [b]P<0.05 compared with KA group; [c]P<0.05 compared with L-CCG group; [d]P<0.05 compared with M-CCG group; [e]P<0.05 compared with H-CCG group.

表3 CCG对IL-1β、TNF-α、IL-6水平的影响

Table 3 Effects of CCG on IL-1β, TNF-α and IL-6 levels

组别 Group	白细胞介素-1β/pg·mL^{-1} IL-1β /pg·mL^{-1}	肿瘤坏死因子-α/pg·mL^{-1} TNF-α /pg·mL^{-1}	白细胞介素-6/pg·mL^{-1} IL-6 /pg·mL^{-1}
HMC3 group	13.62±1.41	62.89±6.75	36.25±3.93
KA group	46.58±4.73[a]	182.07±18.53[a]	96.51±10.36[a]
L-CCG group	37.15±3.86[b]	149.16±15.39[b]	80.17±8.58[b]
M-CCG group	28.37±2.95[bc]	113.28±13.31[bc]	65.79±6.65[bc]
H-CCG group	20.14±2.20[bcd]	88.45±10.26[bcd]	47.62±4.91[bcd]
H-CCG+colivelin group	42.91±4.34[e]	156.24±16.77[e]	89.35±9.14[e]

$\bar{x}\pm s$, n=6; [a]P<0.05, 与HMC3组比; [b]P<0.05, 与KA组比; [c]P<0.05, 与L-CCG组比; [d]P<0.05, 与M-CCG组比; [e]P<0.05, 与H-CCG组比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with HMC3 group; [b]P<0.05 compared with KA group; [c]P<0.05 compared with L-CCG group; [d]P<0.05 compared with M-CCG group; [e]P<0.05 compared with H-CCG group.

平增加(P<0.05); 与KA组比, L-CCG组、M-CCG组、H-CCG组$NADP^+$/NADPH水平依次降低(P<0.05); 与H-CCG组比, H-CCG+colivelin组$NADP^+$/NADPH水平增加(P<0.05)(表2)。

2.7 CCG对IL-1β、TNF-α、IL-6水平的影响

与HMC3组比, KA组HMC3细胞IL-1β、TNF-α、IL-6水平增加 (P<0.05); 与KA组比, L-CCG组、M-CCG组、H-CCG组IL-1β、TNF-α、IL-6水平依次降低(P<0.05); 与H-CCG组比, H-CCG+colivelin组IL-1β、TNF-α、IL-6水平增加(P<0.05)(表3)。

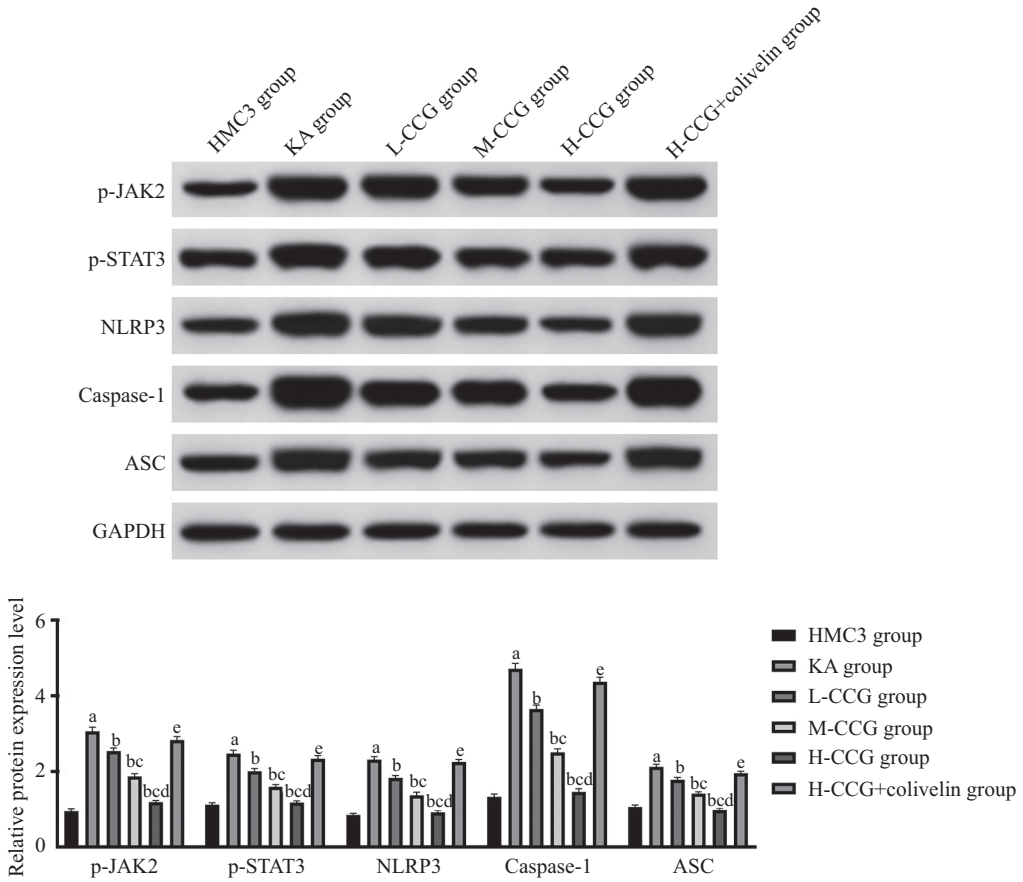
2.8 CCG对p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达的影响

与HMC3组比, KA组HMC3细胞中p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达水平增加(P<0.05); 与KA组比, L-CCG组、M-CCG组、H-CCG组p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达水平依次降低 (P<0.05); 与H-CCG组比, H-CCG+colivelin组p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达水平增加(P<0.05)(图5)。

3 讨论

氧化应激、炎症是癫痫病理学中的重要机制, 参与癫痫发作过程。研究表明, 氧化应激会改变Ca^{2+}体内平衡, 诱发神经系统改变, 加速神经退行性病变和癫痫发作, 而脑部炎症会导致血脑屏障中免疫因子和小胶质细胞产生炎症分子, 导致神经元损伤, 进而促进癫痫发生[17]。近年来, CCG已被证明可以降低氧化应激标志物和促炎因子水平, 减少许多疾病的氧化应激和炎症反应[18]。小胶质细胞是大脑神经发育、神经系统稳态以及急性和慢性脑病理的重要参与者。目前, 小胶质细胞在癫痫方面的研究较多, 而在实验研究中, 癫痫发展通常用KA来进行模拟[19]。因此, 本研究以小胶质细胞为研究对象, 探究CCG对KA诱导的小胶质细胞损伤的影响, 发现KA处理能加重炎症和氧化应激, 促使小胶质细胞损伤。而CCG可有效控制炎症, 减轻氧化应激, 提高增殖率, 进而保护小胶质细胞。

JAK2/STAT3通路与细胞存活和凋亡等基因表达相关, 参与神经系统疾病和免疫炎症性疾病的



$\bar{x}\pm s$, n=6; [a]P<0.05, 与HMC3组比; [b]P<0.05, 与KA组比; [c]P<0.05, 与L-CCG组比; [d]P<0.05, 与M-CCG组比; [e]P<0.05, 与H-CCG组比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with HMC3 group; [b]P<0.05 compared with KA group; [c]P<0.05 compared with L-CCG group; [d]P<0.05 compared with M-CCG group; [e]P<0.05 compared with H-CCG group.

图5 CCG对p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达的影响

Fig.5 Effects of CCG on the expression of p-JAK2, p-STAT3, NLRP3, Caspase-1 and ASC

发展[20]。JAK2/STAT3信号通路能通过缺血刺激参与炎症的发生, 其能调控小胶质细胞/巨噬细胞极化, 减轻脑损伤[21]。激活JAK2/STAT3通路, 可介导小胶质细胞向M1型极化, 加速促炎因子释放和神经元丢失, 最终导致海马神经元炎症损伤[22]。失活JAK2/STAT3通路, 可抑制炎症和凋亡蛋白表达, 减弱小胶质细胞和星形胶质细胞的活化作用, 增加存活神经元的数量, 保护癫痫诱导的脑损伤[23]。阻滞JAK2/STAT3信号转导可抑制小胶质细胞和星形胶质细胞的活化, 促进小胶质细胞极化, 减轻炎症, 延缓神经损伤[24-26]。而IL-6可激活JAK2-STAT3通路, 促进神经胶质细胞过度免疫反应, 从而引发细胞毒性损伤和氧化应激损伤, 导致神经元坏死[27]。本研究发现, KA处理后HMC3细胞中p-JAK2、p-STAT3、NLRP3、Caspase-1、ASC表达水平增加。Caspase-1、ASC及JAK2/STAT3通路能参与激活NLRP3炎症小体, 进而催化炎症级联反应[28], 表明KA可能通过增加炎症反应从而导致小胶质细胞损伤。CCG可通过调节炎症相关基因表达, 有效改善KA介导的小胶质细胞炎症损伤。本研究还表明JAK2/STAT3通路激活剂colivelin能逆转CCG对小胶质细胞的保护作用, 表明CCG对小胶质细胞的影响可能与JAK2/STAT3通路有关。

综上所述, CCG可能通过抑制JAK2/STAT3通路对KA诱导的小胶质细胞功能损伤具有保护作用。这些发现支持了CCG可能成为治疗癫痫的有效和安全的治疗干预措施的观点。然而, 本研究不足的是机制探讨不充分, 且仙茅苷对小胶质细胞的长期影响及潜在副作用也未研究。此外, 在使用CCG作为不同慢性疾病的神经保护剂进行新的临床试验之前, 体外和体内的临床前研究是必不可少的, 故未来将通过相关体内外实验验证CCG对癫痫的治疗作用

和具体作用机制。

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