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• 论著 •

灯盏花乙素调节 AMPK/SIRT1/PGC-1 α 信号通路对溃疡性结肠炎大鼠肠道菌群的影响

吴军城*, 赵立国, 张立文

(博鳌一龄生命养护中心消化内镜, 海南琼海 571400)

【摘要】 目的 探究灯盏花乙素(SCU)调节腺苷酸激活蛋白激酶(AMPK)/沉默调节蛋白1(SIRT1)/过氧化物酶体增殖活化受体 γ 辅助活化因子1 α (PGC1 α)信号通路对溃疡性结肠炎(UC)大鼠肠道菌群的影响。方法 随机选择6只SD大鼠作为空白对照组(NC组),另取24只腹腔注射3.5%葡聚糖硫酸钠(DSS)构建UC大鼠模型。将UC大鼠随机平分为UC组、SCU组(100 mg/kg)、Compound C组(AMPK/SIRT1/PGC-1 α 信号通路抑制剂Compound C 250 μ g/kg体重)以及SCU+Compound C组(SCU100 mg/kg体重+250 μ g/kg体重Compound C),NC组、UC组给予等量生理盐水。每组均6只大鼠。ELISA法检测血清炎症因子水平;HE染色观察结肠组织病理学变化;对粪便进行短链脂肪酸(SCFAs)测序以及16s测序,并进行序列分析;Western blot检测AMPK/SIRT1/PGC-1 α 信号通路蛋白表达水平。结果 NC组大鼠结肠绒毛形态完整,结肠黏膜上皮结构清晰可见,肠腺内多个细胞整齐排列,无任何病变。与NC组相比,UC组大鼠结肠组织隐窝和杯状细胞消失,炎症细胞浸润增加,乙酸盐、丙酸盐、丁酸盐含量,Shannon、Simpson、Chao1指数,乳酸杆菌属相对丰度及AMPK、SIRT1、PGC1 α 水平均显著降低(均 $P<0.05$);IL-1 β 、TNF- α 、IL-6水平,大肠埃希菌-志贺菌属及拟杆菌属相对丰度显著升高(均 $P<0.05$)。与UC组相比,SCU组粘膜结构以及炎症细胞浸润现象得到改善,粘膜结构基本完整,杯状细胞数量显著增多;乙酸盐、丙酸盐、丁酸盐含量,Shannon、Simpson、Chao1指数,乳酸杆菌属相对丰度及AMPK、SIRT1、PGC1 α 水平均显著增加(均 $P<0.05$);IL-1 β 、TNF- α 、IL-6水平,大肠埃希菌-志贺菌属及拟杆菌属相对丰度均显著减少(均 $P<0.05$)。Compound C组上述指标变化趋势与SCU组比较均相反。结论 SCU可通过激活AMPK/SIRT1/PGC-1 α 信号轴升高有益菌的丰度,降低致病菌,改善UC大鼠肠道菌群失衡,减轻UC的炎症状态,发挥对UC的治疗作用。

【关键词】 灯盏花乙素;AMPK/SIRT1/PGC-1 α 信号通路;肠道菌群;溃疡性结肠炎

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Regulatory effect of Scutellarin B on the gut microbiota of ulcerative colitis rats by regulating AMPK/SIRT1/PGC-1 α signaling pathway

WU Juncheng, ZHAO Ligu, ZHANG Liwen (Digestive Endoscopy, Boao Yiling Life Care Center, Qionghai 571400, Hainan, China)*

【Abstract】 **Objective** To investigate the effect of Scutellarin B (SCU) on the gut microbiota of ulcerative colitis (UC) rats by regulating the adenosine monophosphate-activated protein kinase (AMPK)/sirtuin 1 (SIRT1)/peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) signaling pathway. **Methods** Six SD rats were randomly selected as blank control group (NC group), and another 24 rats were intraperitoneally injected with 3.5% sodium dextran sulfate (DSS) to construct UC rat model. UC rats were randomly divided into UC group, SCU group (100 mg/kg), Compound C group (AMPK/SIRT1/PGC-1 α signaling pathway inhibitor Compound C 250 μ g/kg body weight), and SCU + Compound C group (SCU 100 mg/kg body weight + 250 μ g/kg body weight Compound C), NC group and UC group were given equal amounts of physiological saline. There were 6 rats in each group. ELISA method was used to detect serum inflammatory factor level; HE staining was used to observe the histopathological changes of colon tissue; short chain fatty acid (SCFAs) sequencing and 16s sequencing were performed on feces and sequence analysis was performed; Western blot was used to detect the expression level of AMPK/SIRT1/PGC-1 α signaling pathway proteins. **Results** The morphology of colonic villi in NC group was intact, and the epithelial structure of colonic mucosa was clearly visible, multiple cells in the intestinal glands were neatly arranged without any lesions. Compared with the NC group, the crypts and goblet cells in the colonic tissue of UC group disappeared, and the infiltration of inflammatory cells increased, the contents of acetate, propionate, and butyrate, the indexes of Shannon, Simpson, and Chao1, the relative abundance of

* **【通讯作者(简介)】** 吴军城(1985-),男,广东河源人,硕士研究生,主治医师,主要从事中医消化方面的研究。E-mail:wcj023456@163.com

Lactobacillus, and the levels of AMPK, SIRT1, and PGC1 were significantly decreased ($P < 0.05$), the levels of IL-1 β , TNF- α , IL-6, and the relative abundances of *Escherichia coli* Shigella and Bacteroidetes were significantly increased ($P < 0.05$). Compared with UC group, the SCU group had improved the mucosal structure and inflammatory cell infiltration were improved, the mucosal structure was basically intact, and the number of goblet cells increased significantly in SCU group, the contents of acetate, propionate, and butyrate, the indexes of Shannon, Simpson, and Chao1, the relative abundance of Lactobacillus, and the levels of AMPK, SIRT1, and PGC1 were significantly increased (all $P < 0.05$), the levels of IL-1 β , TNF- α , IL-6, and the abundances of *E. coli* Shigella and Bacteroidetes were significantly decreased (all $P < 0.05$). The change trend of the above indexes in Compound C group was opposite to that in SCU group. **Conclusion** SCU can increase the abundance of beneficial bacteria by activating the AMPK/SIRT1/PGC-1 α signaling axis, reduce pathogenic bacteria, improve the imbalance of gut microbiota in UC rats, reduce the inflammatory state of UC, and play a therapeutic role in UC.

【Key words】 Scutellarin B; AMPK/SIRT1/PGC-1 α signaling pathway; Intestinal microbiota; Ulcerative colitis

溃疡性结肠炎(UC)是一种由宿主肠道微生物群失衡引起的结肠慢性炎症性疾病,腹泻腹痛为主要症状^[1]。尽管近年来在UC的诊断和治疗方面取得了很大进展,但仍缺乏有效治疗药物^[2-3]。UC的发生通常与肠道微生物群紊乱有关^[4]。因此,改善肠道菌群对于治疗UC具有重要意义。灯盏花乙素(SCU)是一种天然类黄酮,已被证实可通过下调促炎细胞因子和抑制细胞凋亡及氧化应激预防UC的发生^[5]。但关于SCU对UC大鼠肠道菌群的影响尚不清楚。腺苷酸激活蛋白激酶(AMPK)/沉默调节蛋白1(SIRT1)/过氧化物酶体增殖活化受体 γ 辅助活化因子1 α (PGC1 α)介导的信号级联反应可抑制活性氧(ROS)和炎性细胞因子的产生,因此认为激活级联反应可能是治疗炎症性肠病的有效疗法^[6]。Zhang等^[7]报道,激活AMPK/SIRT1/PGC-1 α 途径可保护肠上皮细胞免受脂多糖诱导的肠道通透性增加、氧化应激、炎症及细胞凋亡,但激活AMPK/SIRT1/PGC-1 α 信号轴是否可对UC大鼠产生积极影响尚不明确。本研究旨在探究SCU是否可通过激活AMPK/SIRT1/PGC-1 α 信号轴对UC大鼠肠道菌群失衡起到改善作用。

材料与方法

1 材料

1.1 实验动物 7周龄SPF级SD雄性大鼠,体重220~235 g,由广州相观生物属技术有限公司提供,动物许可证号SCXK(粤)2021-0058。本试验得到本院动物伦理委员会的批准。

1.2 主要试剂 SCU购自上海源叶生物属技术有限公司;葡聚糖硫酸钠(DSS)购自北京康瑞纳生物属技术有限公司;AMPK/SIRT1/PGC-1 α 信号通路抑制剂Compound C购自美国MedChemExpress LLC;肿瘤坏死因子 α (TNF- α)ELISA试剂盒购自上海酶研生物属技术有限公司;白介素(IL)-1 β 、IL-6 ELISA试剂盒购自武汉菲恩生物属技术有限公司;AMPK、SIRT1、PGC-

1 α 一抗购自英国Abcam公司。

2 方法

2.1 动物建模及分组 随机选择6只大鼠作为空白对照组(NC组),其余大鼠连续10 d通过腹腔注射3.5% DSS水溶液,建立UC模型^[8]。NC组腹腔注射等量生理盐水。

将UC模型大鼠随机平分为UC组、SCU组、Compound C组以及SCU+Compound C组,每组均6只。SCU组大鼠按100 mg/kg体重的剂量灌胃SCU^[9],并通过尾静脉注射等量生理盐水。Compound C组尾静脉注射Compound C 250 μ g/kg体重^[10],并灌胃等量生理盐水。SCU+Compound C组灌胃SCU 100 mg/kg体重,同时尾静脉注射Compound C 250 μ g/kg体重。NC组、UC组给予等量生理盐水。每天给药1次,连续治疗2周。

2.2 标本采集 药物治疗后,采用腹部按摩法采集大鼠粪便,用于16S rDNA测序和短链脂肪酸(SCFAs)含量测定。大鼠禁食24 h,然后用2%戊巴比妥钠麻醉。从腹主动脉采血样,分离血清,用于细胞因子检测。颈椎脱臼处死大鼠,取结肠并分为两段:一段用4%多聚甲醛固定后作组织病理学检查;另一段用于AMPK/SIRT1/PGC-1 α 信号通路蛋白检测。

2.3 血清细胞因子检测 取在鼠血清,采用ELISA法检测IL-1 β 、TNF- α 、IL-6水平,按试剂盒说明书操作。

2.4 结肠组织病理学检查 取4%多聚甲醛固的大鼠结肠组织,用去离子水洗涤30 min,依次用75%、85%、95%和100%乙醇脱水,再将结肠组织浸泡在二甲苯溶液中进行透化。然后将结肠组织有石蜡包埋并切片(厚度5 μ m),苏木精/伊红(H&E)染色,显微镜下观察结肠组织病理学变化。

2.5 粪便SCFAs含量测定 取大鼠粪便0.5 g,悬浮在2 mL甲醇中,并用硫酸溶液将pH值调节至2.0;将悬浮液冰浴20 min,并使用涡流混合器立即均匀

化,4℃、12 000 g 离心 15 min,取上清液过滤,使用 Shimadzu GC2010A 气相色谱仪器与 MS-QP2010 质谱仪耦合测定 SCFAs 的含量。起始温度设定为 220℃,将 1.0 μL 样品注入 GC-MS 系统。每个样品的分析运行时间设定为 17.5 min。检测条件为氮气,流速为 1.0 mL/min,电离电压 70 eV,入口温度 220℃,探测器温度为 250℃。以样品浓度为横轴,样品质谱峰面积为纵轴绘制标准曲线,通过标准曲线计算各组大鼠 SCFAs 含量。

2.6 粪便 16srDNA 扩增及测序分析 使用 FastDNA Spin 试剂盒提取大鼠结肠内容物 DNA。使用对应于 V3-V4 区域的引物(正向:5'-CTTAYGG GRBGCASCAG-3';反向:5'-GGACTACHVGGGT WTCTAAT-3')扩增 16S rDNA。反应体系:5×FastPfu Buffer 4 μL、2.5 mmol/L dNTPs 2 μL、正向引物 0.8 μL、反向引物 0.8 μL、FastPfu Polymerase 0.4 μL、10 ng 模板 2 μL、加 ddH₂O 至 20 μL。反应程序:95℃预变性 1 min,95℃变性 10 s,50℃退火 30 s,72℃延伸 60 s,共 30 个循环。使用 Invitrogen 的 Quant-iT[®] dsDNA 分析试剂盒对扩增产物进行定量。使用 Illumina MiSeq 测序平台进行 2×150 bp 双向全基因组测序,以 97% 的相似性聚类成 OTU,以 OTU 为基本单位分析菌群组成。

2.7 AMPK/SIRT1/PGC-1α 信号通路蛋白检测 采用 Western blot 法。将大鼠结肠组织在液氮中研磨,用 RIPA 裂解液提取总蛋白,使用 BCA 试剂盒定量蛋白质浓度。取 30 mg 裂解物经 SDS-PAGE 电泳后转印到聚偏二氟乙烯膜上,室温下用 5% 脱脂乳封闭 1 h;分别加入一抗 AMPK (1:1000)、SIRT1 (1:1000)、PGC-1α (1:500) 和 GAPDH 抗体 (1:1000),室温孵育,TBST 洗涤;加入辣根过氧化物酶 (HRP) 偶联的二抗,室温孵育 1 h,洗涤后使用 ECL 试剂进行可视化。使用 ImageJ 软件对条带灰度值进行分析。

2.8 统计学分析 应用 8.0 版 GraphPad Prism 软件进行处理数据。实验数据经正态分布检验后用 ($\bar{x} \pm s$) 描述,多组间比较采用单因素方差分析,两两比较采用 SNK-*q* 检验。 $P < 0.05$ 为差异有统计学意义。

结果

1 SCU 对大鼠血清 IL-1β、TNF-α、IL-6 水平的影响

采用 ELISA 检测大鼠血清细胞因子水平,结果见表 1。与 NC 组相比,UC 组大鼠血清 IL-1β、TNF-α、IL-6 水平均显著升高 ($t = 9.122, 11.562, 11.979$, 均 $P < 0.05$);与 UC 组相比,SCU 组 IL-1β、TNF-α、IL-6 水平均显著下降 ($t = 6.199, 9.093, 9.135$, 均 $P < 0.05$),Compound C 组 IL-1β、TNF-α、IL-6 水平均显

著升高 ($t = 10.337, 11.257, 10.835$, 均 $P < 0.05$);与 SCU 组相比,SCU + Compound C 组 IL-1β、TNF-α、IL-6 水平均显著升高 ($t = 5.648, 8.495, 5.072$, 均 $P < 0.05$)。

表 1 各组大鼠细胞因子比较 ($\bar{x} \pm s$, pg/mL)
Table 1 Comparison of cytokines in each group

组别 Group	IL-1β	TNF-α	IL-6
NC 组 (n=6)	22.01±2.34	7.85±1.13	10.54±2.56
UC 组 (n=6)	34.93±3.38 ^a	18.48±2.75 ^a	28.23±3.98 ^a
SCU 组 (n=6)	26.15±2.23 ^b	10.12±1.54 ^b	14.74±2.35 ^b
Compound C 组 (n=6)	49.57±5.07 ^b	28.83±3.34 ^b	44.23±5.35 ^b
SCU+Compound C 组 (n=6)	34.15±3.55 ^c	17.93±1.73 ^c	22.23±2.98 ^c

注:a 与 NC 组比较, $P < 0.05$;b 与 UC 组比较, $P < 0.05$;c 与 SCU 组比较, $P < 0.05$ 。

2 SCU 对大鼠结肠组织病理学变化的影响

图 1 显示,NC 组大鼠结肠绒毛形态完整,结肠黏膜上皮结构清晰可见,肠腺内多个细胞整齐排列,无任何病变;与 NC 组相比,UC 组隐窝和杯状细胞消失,炎症细胞浸润增加;与 UC 组相比,SCU 组黏膜结构以及炎症细胞浸润现象得到改善,黏膜结构基本完整,杯状细胞数量显著增加,而 Compound C 组大鼠组织损伤加重;SCU + Compound C 组与 UC 组结构相似。

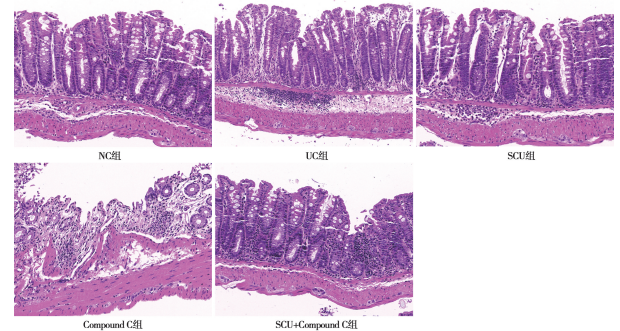


图 1 大鼠结肠组织病理学变化 (HE 染色, 200×)
Fig. 1 Histopathological changes of colon in rats (HE staining, 200×)

3 SCU 对大鼠粪便 SCFAs 含量的影响

与 NC 组相比,UC 组大鼠乙酸盐、丙酸盐、丁酸盐含量均显著降低 ($t = 10.749, 9.549, 10.379$, 均 $P < 0.05$);与 UC 组相比,SCU 组乙酸盐、丙酸盐、丁酸盐含量均显著升高 ($t = 7.469, 6.629, 7.948$, 均 $P < 0.05$),Compound C 组乙酸盐、丙酸盐、丁酸盐含量均显著降低 ($t = 11.985, 13.815, 8.835$, 均 $P < 0.05$);与 SCU 组相比,SCU + Compound C 组乙酸盐、丙酸盐、丁酸盐含量均显著降低 ($t = 5.864, 4.145, 4.819$, 均 $P < 0.05$) (表 2)。

4 SCU 对大鼠肠道菌群的影响

与 NC 组相比,UC 组 Shannon、Simpson、Chao1 指数均显著降低 ($t = 11.679, 18.647, 9.041$, 均 $P <$

0.05);与UC组相比,SCU组Shannon、Simpson、Chao1指数均显著升高($t=10.689, 14.296, 6.342$,均 $P<0.05$),Compound C组Shannon、Simpson、Chao1指数均显著降低($t=9.897, 7.754, 8.119$,均 $P<0.05$);与SCU组相比,SCU+Compound C组Shannon、Simpson、Chao1指数均显著降低($t=9.369, 12.842, 5.729$,均 $P<0.05$)(表3)。

表2 SCU对大鼠粪便SCFAs含量的影响($\bar{x}\pm s, \mu\text{g/mL}$)
Table 2 Effect of SCU on SCFAs content in rat stool

组别 Group	乙酸盐 Acetate	丙酸盐 Propionate	丁酸盐 Butyrate
NC组($n=6$)	77.63±8.07	96.27±9.92	190.15±23.01
UC组($n=6$)	51.18±5.09 ^a	65.47±7.95 ^a	120.31±14.04 ^a
SCU组($n=6$)	69.56±6.14 ^b	86.85±9.59 ^b	173.79±18.44 ^b
Compound C组($n=6$)	21.69±2.18 ^b	20.91±2.12 ^b	60.86±7.09 ^b
SCU+Compound C组($n=6$)	55.13±6.91 ^c	73.48±7.35 ^c	141.36±15.54 ^c

注:a与NC组比较, $P<0.05$;b与UC组比较, $P<0.05$;c与SCU组比较, $P<0.05$ 。

表3 SCU对大鼠肠道菌群 α 多样性的影响($\bar{x}\pm s, \mu\text{g/mL}$)
Table 3 Effect of SCU on α diversity of intestinal flora in rats

组别 Group	Shannon	Simpson	Chao1
NC组($n=6$)	5.22±0.53	1.44±0.17	431.67±45.29
UC组($n=6$)	3.45±0.20 ^a	0.67±0.05 ^a	303.73±32.48 ^a
SCU组($n=6$)	5.07±0.48 ^b	1.26±0.12 ^b	393.48±36.58 ^b
Compound C组($n=6$)	1.95±0.17 ^b	0.35±0.02 ^b	188.84±22.57 ^b
SCU+Compound C组($n=6$)	3.65±0.33 ^c	0.73±0.07 ^c	312.40±32.47 ^c

注:a与NC组比较, $P<0.05$;b与UC组比较, $P<0.05$;c与SCU组比较, $P<0.05$ 。

与NC组相比,UC乳酸杆菌属相对丰度显著降低($t=13.151, P<0.05$),大肠埃希菌-志贺菌属、拟杆菌属相对丰度显著升高($t=11.731, 14.524, P<0.05$)。与UC组相比,SCU乳酸杆菌属相对丰度显著升高($t=9.393, P<0.05$),大肠埃希菌-志贺菌属、拟杆菌属相对丰度均显著降低($t=8.971, 11.619$,均 $P<0.05$);Compound C乳酸杆菌属相对丰度显著降低($t=13.151, P<0.05$),大肠埃希菌-志贺菌属、拟杆菌属相对丰度显著升高($t=9.661, 13.555, P<0.05$)。与SCU组相比,SCU+Compound C组乳酸杆菌属相对丰度显著降低($t=6.575, P<0.05$),大肠埃希菌-志贺菌属、拟杆菌属相对丰度均显著升高($t=5.521, 12.587$,均 $P<0.05$)(表4,图2)。

5 SCU对大鼠结肠组织AMPK/SIRT1/PGC1 α 表达水平的影响

与NC组相比,UC组大鼠结肠组织AMPK、SIRT1、PGC1 α 水平均显著下降($t=17.160, 23.866, 9.580$,均 $P<0.05$);与UC组相比,SCU组大鼠结肠组织AMPK、SIRT1、PGC1 α 水平均显著升高($t=13.547, 17.398, 7.185, P<0.05$,Compound C组大

鼠结肠组织AMPK、SIRT1、PGC1 α 水平均显著下降($t=6.322, 5.799, 8.862$,均 $P<0.05$);与SCU组相比,SCU+Compound C组大鼠结肠组织AMPK、SIRT1、PGC1 α 水平均显著下降($t=11.440, 15.837, 5.748, P<0.05$)(表5,图3)。

表4 SCU对大鼠属水平优势菌群相对丰度的影响($\bar{x}\pm s$)
Table 4 Effect of SCU on the relative abundance of dominant bacteria at genus level in rats

分组 Group	乳酸杆菌 Lactobacillus	拟杆菌 Bacteroidetes	大肠埃希菌-志贺菌 Escherichia-Shigella
NC组($n=6$)	0.32±0.04	0.20±0.02	0.11±0.01
UC组($n=6$)	0.18±0.02 ^a	0.37±0.05 ^a	0.26±0.03 ^a
SCU组($n=6$)	0.28±0.03 ^b	0.24±0.03 ^b	0.14±0.02 ^b
Compound C组($n=6$)	0.04±0.01 ^b	0.51±0.04 ^b	0.40±0.03 ^b
SCU+Compound C组($n=6$)	0.21±0.02 ^c	0.32±0.03 ^c	0.27±0.03 ^c

注:a与NC组比较, $P<0.05$;b与UC组比较, $P<0.05$;c与SCU组比较, $P<0.05$ 。

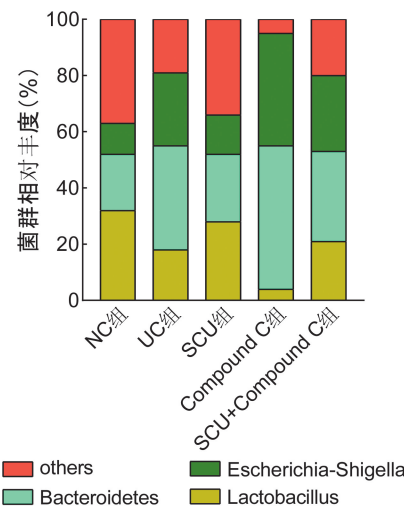


图2 各组大鼠属水平优势菌群的相对丰度的比较
Fig. 2 Comparison of relative abundance of genus level dominant flora in rats in each group

表5 各组大鼠结肠组织AMPK/SIRT1/PGC1 α 蛋白表达水平比较($\bar{x}\pm s$)
Table 5 Comparison of AMPK/SIRT1/PGC1 α expression levels in colon tissues of rats in all groups

组别 Group	AMPK/ GAPDH	SIRT1/ GAPDH	PGC1 α / GAPDH
NC组($n=6$)	0.89±0.10	1.65±0.16	1.21±0.13
UC组($n=6$)	0.32±0.03 ^a	0.58±0.08 ^a	0.81±0.12 ^a
SCU组($n=6$)	0.77±0.14 ^b	1.36±0.15 ^b	1.11±0.11 ^b
Compound C组($n=6$)	0.11±0.01 ^b	0.32±0.03 ^b	0.44±0.05 ^b
SCU+Compound C组($n=6$)	0.39±0.05 ^c	0.65±0.07 ^c	0.87±0.08 ^c

注:a与NC组比较, $P<0.05$;b与UC组比较, $P<0.05$;c与SCU组比较, $P<0.05$ 。

讨论

UC是一种炎症性肠病,其特征是肠黏膜损伤和炎症,从直肠开始延伸至结肠近端,主要临床表现为腹痛、黏液、腹泻和血便^[11]。UC的发病机制与宿主遗传、炎症过度反应和微生物失衡有关^[12]。Shao等^[13]报道有20%~30%的UC患者需要手术,给家庭带来

巨大的经济负担。治疗 UC 的药物主要包括类固醇、氨基水杨酸盐、免疫抑制剂和生物制剂^[14]。然而这些药物有严重的副作用,包括引起感染、发热、腹泻和高复发率等^[15]。因此,亟待寻找一种新的、安全的方法用于治疗 UC。

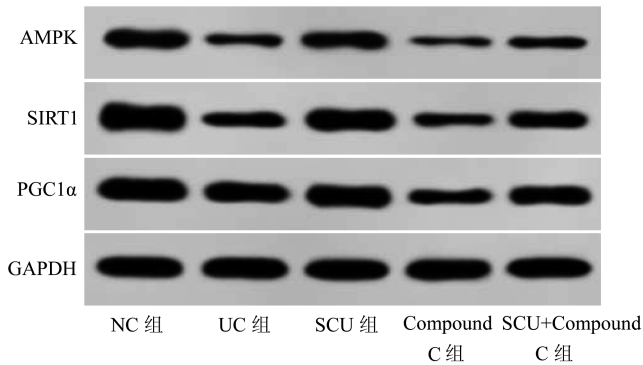


图 3 Western blot 检测大鼠结肠组织 AMPK、SIRT1、PGC1α 蛋白
Fig. 3 Western blot analysis of AMPK, SIRT1 and PGC1α in rat colon tissue

SCU 是一种从灯盏花中提取的黄酮苷,具有多种病理特性,如具有抗炎、抗氧化及血管调节作用。SCU 可抑制由 DSS 引起的小鼠结肠炎相关癌症的癌变,并缓解病理症状^[16]。Zhou 等^[17]报道百里香多酚通过减轻肠道屏障损伤,调节肠道微生物群和抑制炎症小体改善 DSS 诱导的小鼠 UC,而百里香多酚的主要活性成分为 SCU。本研究观察了 SCU 对 UC 大鼠肠道菌群的影响。结果表明,在 UC 大鼠,结肠组织隐窝和杯状细胞消失,炎症细胞浸润增加,而 SCU 可改善 UC 大鼠肠粘膜结构以及炎症细胞浸润现象,粘膜结构基本完整,杯状细胞数量显著增加,显示 SCU 对 UC 大鼠肠道损伤起到改善效果。

肠道微生物群在能量产生、营养供应、宿主防御和免疫力发展中起重要作用^[18]。肠道菌群的改变会影响人体的正常生理功能,导致多种肠道疾病的发生^[19]。大量研究表明,UC 的发生发展与肠道菌群失衡有关,UC 患者肠道菌群失衡主要表现为细菌多样性减少、益生菌减少和致病菌增加^[20]。乳酸杆菌是一种益生菌,在治疗炎症性肠病方面显示出潜在活性^[21],而大肠埃希菌-志贺菌属为一种促进肠道炎症的细菌^[22]。对人和小鼠肠道微生物群的研究表明,增加乳酸杆菌和双歧杆菌等有益细菌,可减少大肠埃希菌-志贺菌属等有害菌,从而缓解 UC 患者的炎症^[23]。本研究中 UC 大鼠肠道菌群 Shannon、Simpson、Chao1 指数及乳酸杆菌属相对丰度显著降低,大肠埃希菌-志贺菌属和拟杆菌属相对丰度显著升高,而 SCU 处理后 Shannon、Simpson、Chao1 指数、乳酸杆菌属相对丰度显著增加,大肠埃希菌-志贺菌属、拟杆

菌属相对丰度显著降低,表明 SCU 可能通过减少有害菌和增加有益菌改善肠道菌群失衡状态,进而对大鼠 UC 起到治疗作用。丁酸有助于保护肠壁,抑制肠道炎症的发生进而增强 UC 的治疗效果。且丁酸盐是结肠上皮细胞的主要能量来源,在维持肠道微生物群稳定性和肠上皮完整性方面起重要作用^[24]。肠道屏障的完整性可抑制炎症因子的释放^[25]。本研究结果显示,UC 大鼠粪便乙酸盐、丙酸盐、丁酸盐含量均显著降低,血清 IL-1β、TNF-α、IL-6 水平显著升高,而 SCU 治疗可使粪便乙酸盐、丙酸盐、丁酸盐含量显著升高,血清 IL-1β、TNF-α、IL-6 水平显著下降,表明 SCU 可能通过增加丁酸盐等 SCFAs 含量,为结肠上皮细胞提供能量,进而改善肠上皮细胞的完整性,抑制有害菌的生长以及炎症因子的释放,促进有益菌生长,从而对 UC 大鼠起到治疗作用。

AMPK/SIRT1/PGC1α 通路是研究炎症的常见通路,激活 AMPK/SIRT1/PGC1α 通路可增加有益菌,抑制有害菌以及炎症,改善衰老相关疾病大鼠后代肠道菌群^[26]。Zhang 等^[7]报道激活 AMPK/SIRT1/PGC-1α 途径可保护肠上皮细胞免受脂多糖诱导的损伤。本研究中 UC 大鼠结肠组织 AMPK、SIRT1、PGC1α 水平显著下降,而 SCU 组 AMPK、SIRT1、PGC1α 水平显著升高,表明 SCU 可能通过激活 AMPK/SIRT1/PGC1α 信号通路减轻 UC 大鼠结肠损伤。AMPK/SIRT1/PGC-1α 信号通路抑制剂干预结果显示,Compound C 加重 UC 大鼠肠炎性损伤,并逆转 SCU 对 UC 大鼠肠道菌群的改善作用。

综上所述,SCU 可能通过激活 AMPK/SIRT1/PGC1α 信号通路改善 UC 大鼠肠道菌群失衡状态,通过升高有益菌的丰度,降低致病菌来减轻 UC 的炎症状态,发挥对 UC 的治疗作用。然而黄酮类药物是否均有治疗大鼠 UC 的作用有待进一步探究。

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