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芍药苷通过β-catenin/c-Myc通路抑制有氧糖酵解缓解小鼠脓毒症相关急性肾损伤

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［**摘要**］ **目的** 探究芍药苷(paeoniflorin,PF)在小鼠脓毒症相关急性肾损伤(sepsis-associated acute kidney injury,SA-AKI)中的作用及机制。**方法** 采用腹腔注射脂多糖(lipopolysaccharide,LPS)的方法构建小鼠SA-AKI模型。将24只6~8周龄的雄性C57BL/6J小鼠(体质量为20~25 g),用随机数字表法分为4组(n=6):对照组(Control组);模型组(LPS组),腹腔注射LPS(15 mg/kg);芍药苷组(LPS+PF组),LPS造模前30 min腹腔注射PF(50 mg/kg);β-catenin特异性激动剂BML284组(LPS+PF+BML284组),LPS造模前30 min,先腹腔注射PF(50 mg/kg),再腹腔注射BML284(10 mg/kg)。采用HE染色观察肾脏组织病理变化并进行Paller评分;采用ELISA检测血清肌酐(serum creatinine,Scr)、中性粒细胞明胶酶相关脂质运载蛋白(neutrophil gelatinase-associated lipocalin,NGAL)和乳酸,以及肾组织己糖激酶2(hexokinase2,HK2)、乳酸脱氢酶A(lactate dehydrogenase A,LDHA)、白细胞介素-1β(interleukin-1β,IL-1β)和白细胞介素18(interleukin-18,IL-18)的含量;Western blot检测总β-连环蛋白(beta-catenin,β-catenin)、磷酸化β-catenin^{Y654}(p-β-catenin^{Y654})、细胞核β-catenin以及c-骨髓细胞瘤癌基因蛋白(c-myelocytomatosis oncogene gene,c-Myc)的表达。**结果** 与Control组比较,LPS组HE染色显示肾脏组织受损,Paller评分升高(P<0.05),血清Scr、NGAL增加(P<0.05),肾组织HK2、LDHA及血清乳酸等有氧糖酵解指标和IL-1β、IL-18含量增多(P<0.05),肾组织总β-catenin、p-β-catenin^{Y654}、细胞核β-catenin以及c-Myc表达上升(P<0.05)。与LPS组相比,LPS+PF组HE染色显示肾脏组织损伤减轻,Paller评分下降(P<0.05),血清Scr、NGAL降低(P<0.05),肾组织HK2、LDHA及血清乳酸等有氧糖酵解指标和IL-1β、IL-18含量减少(P<0.05),肾组织总β-catenin、p-β-catenin^{Y654}、细胞核β-catenin以及c-Myc表达下调(P<0.05)。与LPS+PF组比较,LPS+PF+BML284组HE染色显示肾脏组织受损加重,Paller评分升高(P<0.05),血清Scr、NGAL增加(P<0.05),肾组织HK2、LDHA及血清乳酸等有氧糖酵解指标和IL-1β、IL-18含量增多(P<0.05),肾组织总β-catenin、p-β-catenin^{Y654}、细胞核β-catenin以及c-Myc表达上升(P<0.05)。**结论** PF可以缓解SA-AKI,其机制可能是通过抑制β-catenin/c-Myc通路,降低肾组织有氧糖酵解水平和炎症反应。

［**关键词**］ 脓毒症相关急性肾损伤;芍药苷;有氧糖酵解;β-catenin/c-Myc通路;BML284

［**中图法分类号**］ R285.5;R631;R692 ［**文献标志码**］ A

Paeoniflorin alleviates sepsis-associated acute kidney injury in mice by inhibiting aerobic glycolysis through the β-catenin/c-Myc pathway

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［**Abstract**］ **Objective** To investigate the role and mechanism of paeoniflorin (PF) in sepsis-

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associated acute kidney injury (SA-AKI) in mice. **Methods** Mouse SA-AKI model was constructed by intraperitoneal injection of 15 mg/kg LPS. Twenty-four male C57BL/6J mice (6~8 weeks old, weighing 20~25 g) were randomly divided into Control group, model group, PF group (intraperitoneally injected with 50 mg/kg PF 30 min before LPS administration), and β-catenin specific agonist BML284 group (10 mg/kg BML284 by intraperitoneal injection after 50 mg/kg PF administration). The renal histopathological changes were observed by HE staining and Paller scoring. ELISA was used to determine the contents of serum creatinine (Scr), neutrophil gelatinase-associated lipocalin (NGAL) and lactate, and renal contents of hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), IL-1β and IL-18. Western blotting was performed to detect the expression of total β-catenin, p-β-catenin^{Y654}, nucleus β-catenin and c-Myc. **Results** Compared with the Control group, the LPS group showed obviously damaged renal tissue, higher Paller score (P<0.05), increased serum Scr and NGAL levels (P<0.05), elevated renal contents of aerobic glycolytic indexes such as HK2, LDHA and serum lactate, as well as contents of IL-1β and IL-18 (P<0.05), and enhanced expression of total β-catenin, p-β-catenin^{Y654}, nucleus β-catenin and c-Myc in the renal tissue (P<0.05). PF treatment attenuated the renal tissue damage, decreased Paller score (P<0.05), reduced serum Scr and NGAL levels (P<0.05), HK2, LDHA and serum lactate levels, and contents of IL-1β and IL-18 in renal tissues (P<0.05), and down-regulated the renal expression of total β-catenin, p-β-catenin^{Y654}, nucleus β-catenin and c-Myc when compared with the levels in the model group (P<0.05). While, addition of BML284 reversed above effects of PF treatment with significant differences (P<0.05). **Conclusion** PF can alleviate SA-AKI, and its mechanism may be through its inhibiting the β-catenin/c-Myc pathway, thus reducing the aerobic glycolysis level and inflammatory response in renal tissue.

[**Key words**] sepsis-associated acute kidney injury; paeoniflorin; aerobic glycolysis; β-catenin/c-Myc; BML284

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脓毒症相关急性肾损伤(sepsis-associated acute kidney injury, SA-AKI)定义为诊断脓毒症后7 d内发生的急性肾损伤[1]。全球每年有多达600万例SA-AKI患者,而在脓毒症患者中SA-AKI的发生率超过60%[2]。SA-AKI的治疗策略主要以治疗原发病、优化血流动力学以及避免应用损伤肾脏的药物等肾脏保护性治疗策略为主,但治疗现状并不乐观[3]。因此,迫切需要研究SA-AKI的发病机制,为其防治策略提供更多理论依据。

在有氧环境下,肿瘤细胞表现出高葡萄糖摄取率,葡萄糖转化的丙酮酸分解代谢为乳酸,而不进行氧化磷酸化,这种代谢方式称为沃伯格效应,即有氧糖酵解[4]。己糖激酶2(hexokinase2, HK2)是有氧糖酵解的第一步限速酶,使葡萄糖磷酸化为葡萄糖-6-磷酸[5];作为有氧糖酵解的终产物,乳酸仅能由乳酸脱氢酶A(lactate dehydrogenase A, LDHA)催化丙酮酸生成[6]。因此,HK2、LDHA和乳酸可作为有氧糖酵解指标,反映有氧糖酵解的程度[7-8]。之后有研究发现,脓毒症发生时,有氧糖酵解迅速增强,驱动巨噬细胞和单核细胞炎症反应[9],在提供能量的同时导致巨噬细胞向促炎型M1巨噬细胞转化[10],产生过度炎症反应[11]及心功能不全[12]等。但有氧糖酵解是否参与SA-AKI,尚未有研究报道。

越来越多的证据表明,β-catenin/c-Myc通路参与调控肿瘤环境下的有氧糖酵解[13]。FANG等[14]证实,炎症诱导的小鼠结直肠癌模型中,β-catenin/c-Myc通路的激活,可以增强有氧糖酵解,加速癌症发生。动脉粥样硬化模型中,β-catenin特异性激动剂BML284可以激活β-catenin/c-Myc通路,加重炎症反应,加速动脉粥样硬化进展[15]。但β-catenin/c-Myc通路是否参与脓毒症环境下的有氧糖酵解,介导SA-AKI的发生,有待进一步探索。

血必净用于ICU脓毒症相关急性呼吸窘迫综合征患者的治疗[16],芍药苷(paeoniflorin, PF)是血必净的主要成分之一,具有一定的抗炎作用[17]。有研究发现,PF可以激活核因子红细胞2相关因子2

(nuclear factor erythroid 2-related factor 2, Nrf2),减少白细胞介素-1β(interleukin-1β, IL-1β)和白细胞介素18(interleukin-18, IL-18)的释放,改善小鼠的急性肺损伤(acute lung injury, ALI)[18]。但PF可否调控有氧糖酵解减轻小鼠SA-AKI,尚缺乏相关文献报道。因此,本研究采用脂多糖(lipopolysaccharide, LPS)腹腔注射构建小鼠SA-AKI模型,探究PF在SA-AKI中的作用及机制,旨在为PF治疗SA-AKI提供理论依据。

1 材料与方法

1.1 实验动物与主要试剂

24只6~8周龄SPF级健康雄性C57BL/6J小鼠,体质量20~25 g,由兰州大学实验动物中心提供[动物许可证号:SCXK(甘)2018-0002]。小鼠正常进食,自由饮水,人工光照和黑暗时间12 h交替。适应性饲养1周后进行实验。LPS购于美国Sigma公司;芍药苷、β-catenin特异性激动剂BML284购于美国MCE公司;二甲基亚砜(DMSO)和磷酸缓冲盐溶液(PBS)购于北京索莱宝科技有限公司;用于检测血清肌酐(serum creatinine, Scr)、中性粒细胞明胶酶相关脂质运载蛋白(neutrophil gelatinase associated lipocalin, NGAL)、血清乳酸、HK2、LDHA、IL-1β和IL-18的ELISA试剂盒购于上海酶联生物科技有限公司;β-连环蛋白(beta-catenin, β-catenin)、磷酸化β-catenin^{Y654}(phosphorylated beta-catenin^{Y654}, p-β-catenin^{Y654})、肌动蛋白(β-actin)抗体购于美国ImmunoWay公司,c-骨髓细胞瘤癌基因蛋白(c-myelocytomatosis oncogene gene, c-Myc)、组蛋白3(Histone H3)抗体购于美国GeneTex公司。实验过程中动物处理措施符合伦理学要求,已获兰州大学第一医院伦理委员会批准(LDYYLL2024-258)。实验期间未有动物死亡。

1.2 实验分组与处理

将24只小鼠按随机数字表法分为4组(n=6):对照组(Control组),相同时间给予等量含DMSO的PBS;模型组(LPS组),腹腔注射LPS(15 mg/kg)[19];芍药苷组(LPS+PF组),LPS造模前30 min腹腔注射PF(50 mg/kg)[20];β-catenin特异性激动剂BML284组(LPS+PF+BML284组),LPS造模前30 min先腹腔注射PF(50 mg/kg),再腹腔注射BML284(10 mg/kg)[21]。依据相关文献[19-21]和本研究预实验结果,选定LPS、PF和BML284的小鼠给药量及给药时间。

1.3 标本采集

国内外关于SA-AKI小鼠模型的研究大多选择在造模后24 h进行取材[21-24],因此本研究也选择在此时间点收集样本。LPS造模24 h后,用戊巴比妥钠(30 mg/kg)麻醉小鼠,将其仰卧固定,去除心前区毛发并消毒。以食指指腹定位其心脏搏动区,穿刺收集足量血液标本。随后吸入CO_2处死小鼠,沿腹正中线剪开腹膜,充分暴露腹腔后取出肾脏。将一侧肾脏组织固定于4%多聚甲醛溶液(用于病理学观察);另一侧肾脏组织于-80 ℃冰箱储存(用于后续实验)。

1.4 苏木精-伊红(HE)染色

取肾脏组织,置于4%多聚甲醛中固定48 h,脱水后石蜡包埋、切片、HE染色,显微镜下观察肾脏组织病理结构改变。由2名对实验不知情的病理老师,根据Paller评分[25]评估肾小管损伤程度:每个肾脏,选取至少10个不同区域的100个肾小管进行评分。分数越高代表损伤越严重(每个肾小管的最大分数为10分),肾小管上皮细胞扁平化记1分,刷状缘损伤记1分,细胞膜泡形成记1或2分,间质水肿记1分,细胞质空泡化记1分,细胞坏死记1或2分,管腔阻塞记1或2分。

1.5 ELISA检测

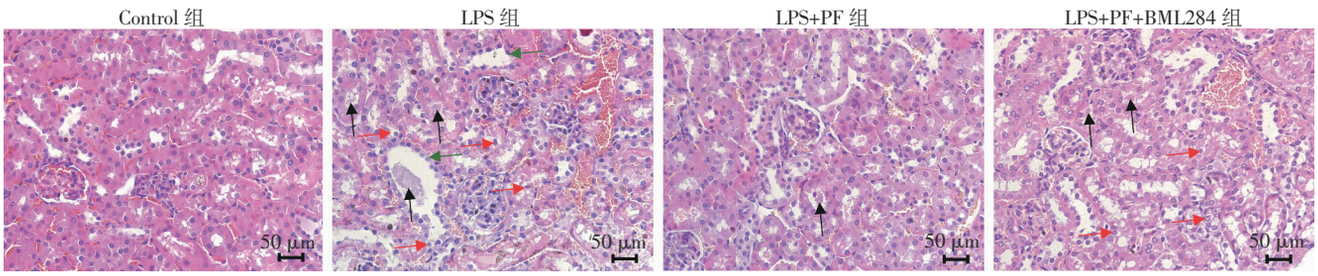
血清:用无菌管收集,室温血液自然凝固15 min,4 ℃、2 500 r/min离心20 min,仔细收集上清液。肾组织:称质量,称取1 g组织,加入9 mL PBS,用匀浆器将标本匀浆充分均匀。将匀浆以4 ℃、1 000×g离心20 min,仔细收集上清液。按照ELISA试剂盒说明书检测血清Scr、NGAL和乳酸,以及肾组织匀浆HK2、LDHA、IL-1β和IL-18水平。

1.6 Western blot检测

取-80 ℃肾组织匀浆,制备蛋白样品。使用10%十二烷基硫酸钠-聚丙烯酰胺凝胶电泳分离,电转移至聚偏二氟乙烯(PVDF)膜上。洗膜后加入β-catenin、p-β-catenin^{Y654}、c-Myc一抗和β-actin、Histone H3内参蛋白(稀释度均为1∶2 000),4 ℃孵育过夜。次日洗膜,使用封闭液按1∶6 000稀释二抗,温室孵育1 h,Tris-HCl缓冲盐溶液(TBST)充分洗膜。置于化学发光仪中曝光,采集图像,ImageJ进行灰度值计算,用目标蛋白与内参蛋白的灰度值比值反映目标蛋白相对表达量。

1.7 统计学分析

采用SPSS 25.0软件进行统计学分析,采用Graphpad Prism 8.0软件作图。实验数据均为计量资料且符合正态分布,以$\bar{x}\pm s$表示,多组间比较采用单因素方差分析,两组间比较采用LSD-t检验。P<0.05为差异有统计学意义。



黑色箭头:管型,绿色箭头:肾小管扩张,红色箭头:肾小管上皮细胞质空泡化

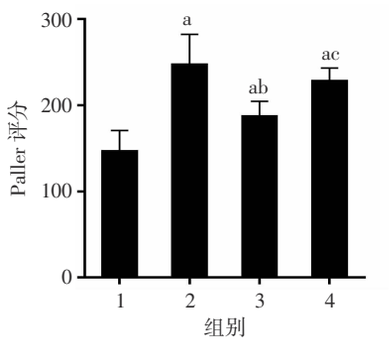
图1　HE染色观察各组小鼠肾组织

2　结果

2.1　各组小鼠肾脏组织病理结构改变

2.1.1　各组小鼠HE病理改变　　光学显微镜下观察Control组肾小管结构清晰,细胞大小形态正常,肾小管管腔内未见管型;与Control组比较,LPS组肾小管扩张、肾小管上皮细胞质空泡化,肾小管管腔内可见大量管型;与LPS组比较,LPS+PF组肾小管扩张减轻,细胞大小形态基本正常,肾小管管腔内少见管型;与LPS+PF组比较,LPS+PF+BML284组肾小管上皮细胞质空泡化,肾小管管腔内可见较多管型,见图1。

2.1.2　各组小鼠肾组织Paller评分　　与Control组比较,LPS组肾组织Paller评分明显增加(P<0.05);与LPS组相比,LPS+PF组肾组织Paller评分明显下降(P<0.05);与LPS+PF组相比,LPS+PF+BML284组肾组织Paller评分明显增加(P<0.05),见图2。



1: Control 组; 2: LPS 组; 3: LPS+PF 组; 4: LPS+PF+BML284 组; a: P<0.05,与 Control 组比较; b: P<0.05,与 LPS 组比较; c: P<0.05,与 LPS+PF 组比较

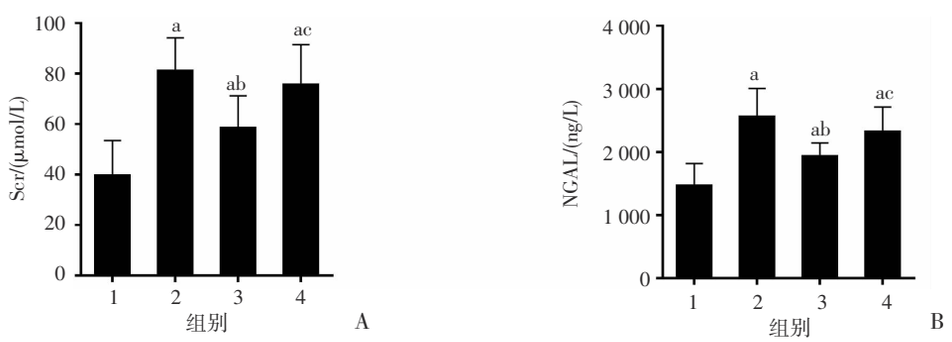
图2　各组小鼠肾脏Paller评分($\bar{x}\pm s$, n=6)

2.2　各组小鼠血清肾损伤标志物Scr、NGAL的含量

ELISA试剂盒检测各组小鼠肾损伤标志物Scr、NGAL的含量。与Control组比较,LPS组中Scr、NGAL含量明显增加(P<0.05);与LPS组比较,LPS+PF组中Scr、NGAL含量明显下降(P<0.05);与LPS+PF组比较,LPS+PF+BML284组中Scr、NGAL含量明显增加(P<0.05)。见图3。

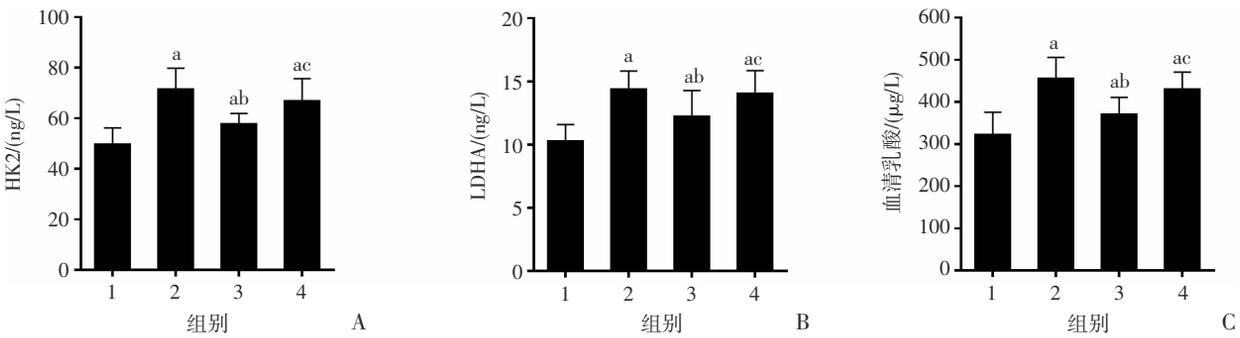
2.3　各组小鼠肾组织HK2、LDHA和血清乳酸等有氧糖酵解指标含量

ELISA试剂盒检测各组小鼠肾组织HK2、LDHA和血清乳酸等有氧糖酵解指标含量。与Control组比较,LPS组中HK2、LDHA和血清乳酸含量明显增加(P<0.05);与LPS组比较,LPS+PF组中HK2、LDHA和血清乳酸含量明显下降(P<0.05);与LPS+PF组比较,LPS+PF+BML284组中HK2、LDHA和血清乳酸含量明显增加(P<0.05)。见图4。



1: Control 组; 2: LPS 组; 3: LPS+PF 组; 4: LPS+PF+BML284 组; a: P<0.05,与 Control 组比较; b: P<0.05,与 LPS 组比较; c: P<0.05,与 LPS+PF 组比较

图3　ELISA法检测各组小鼠血清肾损伤标志物Scr(A)、NGAL(B)的含量($\bar{x}\pm s$, n=6)



1：Control组；2：LPS组；3：LPS+PF组；4：LPS+PF+BML284组；a：P<0.05，与Control组比较；b：P<0.05，与LPS组比较；c：P<0.05，与LPS+PF组比较

图4 ELISA法检测各组小鼠肾组织HK2（A）、LDHA（B）和血清乳酸（C）有氧糖酵解指标含量（$\bar{x}\pm s$，n=6）

2.4 各组小鼠肾组织炎症因子IL-1β、IL-18的含量

ELISA试剂盒检测各组小鼠肾组织炎症因子IL-1β、IL-18的含量。与Control组相比，LPS组中IL-1β、IL-18含量明显增加（P<0.05）；与LPS组相比，LPS+PF组中IL-1β、IL-18含量明显下降（P<0.05）；与LPS+PF组相比，LPS+PF+BML284组中IL-1β、IL-18含量明显增加（P<0.05），见图5。

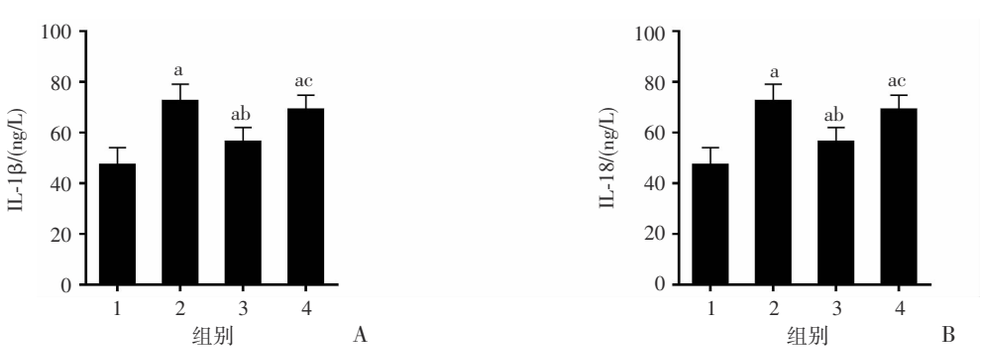
2.5 各组小鼠肾组织β-catenin/c-Myc通路蛋白表达水平

Western blot检测各组小鼠肾组织总β-catenin、p-β-catenin^{Y654}、细胞核β-catenin以及c-Myc蛋白表达水平。与Control组相比，LPS组中总β-catenin、p-β-catenin^{Y654}、细胞核β-catenin以及c-Myc蛋白表达明显增加（P<0.05）；与LPS组相比，LPS+PF组中总β-catenin、p-β-catenin^{Y654}、细胞核β-catenin以及c-Myc蛋白表达明显下降（P<0.05）；与LPS+PF组相比，LPS+PF+BML284组中总β-catenin、p-β-catenin^{Y654}、细胞核β-catenin以及c-Myc蛋白表达明显增加（P<0.05），见图6。

3 讨论

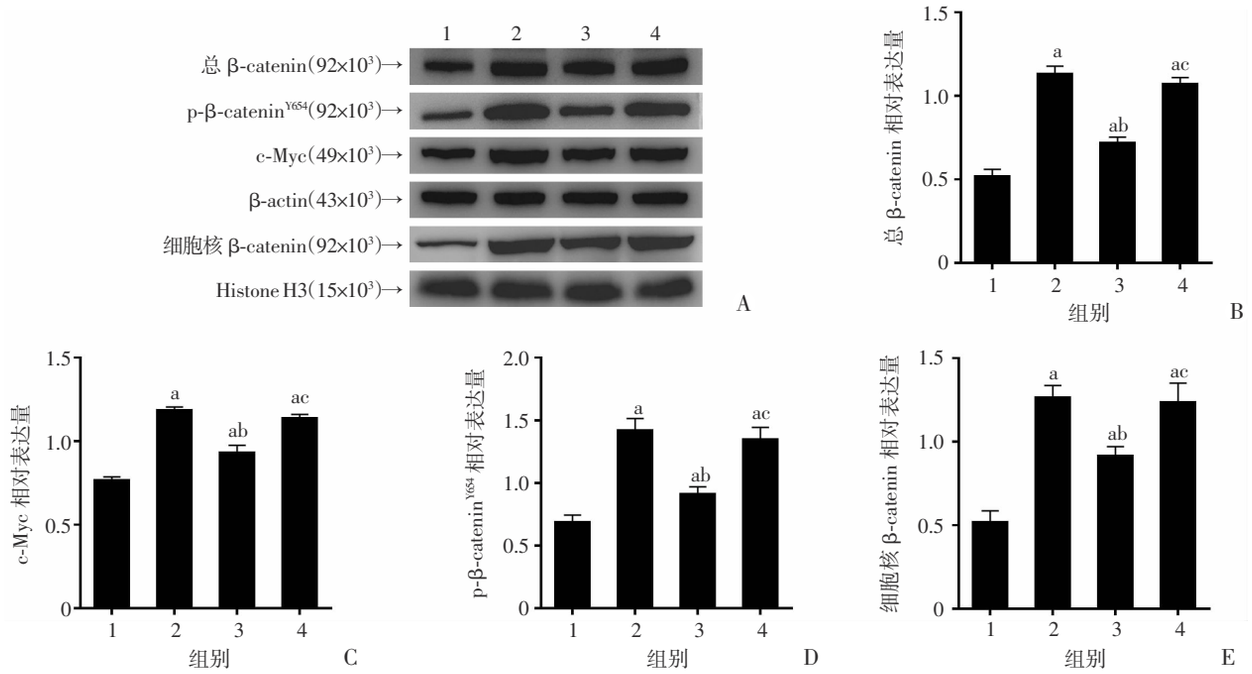
本实验通过腹腔注射LPS构建小鼠SA-AKI模型。LPS组造模24 h后，小鼠出现精神萎靡、活动减少且迟缓、对声音无反应、触碰后呈震颤样缓慢移动、背部竖毛、眼角有明显分泌物、呼吸频率明显下降和间断性喘气等症状，肾组织Paller评分升高，肾损伤标志物Scr、NGAL的含量增加，肾脏炎症反应加重，证实小鼠SA-AKI模型制备成功。

研究发现，在光学显微镜下，SA-AKI中95%肾脏病理改变为肾小管细胞刷状缘消失、间质水肿、细胞质空泡化和管腔阻塞等，而肾小球结构几乎没有受到影响[26]。因此，本研究采用Paller评分量化肾小管损伤程度，反映肾脏病理损伤状况。Scr是一种普遍使用的肾损伤标志物，在评估肾损伤方面具有重要意义[27]。NGAL是一种在肾小管上皮细胞中表达的蛋白，在肾损伤3 h后，血液即可检测到NGAL，其敏感性高于其他传统肾损伤标志物[28]。因此，本研究选取Scr和NGAL作为肾损伤标志物。



1：Control组；2：LPS组；3：LPS+PF组；4：LPS+PF+BML284组；a：P<0.05，与Control组比较；b：P<0.05，与LPS组比较；c：P<0.05，与LPS+PF组比较

图5 ELISA法检测各组小鼠肾组织炎症因子IL-1β（A）、IL-18（B）的含量（$\bar{x}\pm s$，n=6）



1: Control组;2:LPS组;3:LPS+PF组;4:LPS+PF+BML284组;a: $P<0.05$,与 Control 组比较;b: $P<0.05$,与 LPS 组比较;c: $P<0.05$,与 LPS+PF 组比较;A: Western blot 检测肾组织 β-catenin/c-Myc 通路蛋白表达水平;B: 总 β-catenin 蛋白相对表达量;C: c-Myc 蛋白相对表达量;D:p-β-catenin^{Y654}蛋白相对表达量;E:细胞核 β-catenin 蛋白相对表达量

图6 Western blot检测各组小鼠肾组织β-catenin/c-Myc通路蛋白表达水平（$\bar{x}\pm s$, n=6）

3.1 小鼠SA-AKI的发生可能与有氧糖酵解的增强和过度炎症反应有关

过度炎症反应是脓毒症发病机制中不可忽视的核心要素[29]。在小鼠SA-AKI模型中,炎症反应明显加重,炎症因子IL-1β、IL-18的释放增加,肾小管上皮细胞及周围毛细血管通透性明显受损[30]。本研究发现,LPS组小鼠肾组织Paller评分上升,肾损伤标志物Scr、NGAL增加,肾组织HK2、LDHA和血清乳酸等有氧糖酵解指标及炎症因子IL-1β、IL-18含量增多。因此,推测小鼠SA-AKI的发生可能与有氧糖酵解的增强和过度炎症反应有关。在生理条件下,机体的代谢方式主要为氧化磷酸化[31],但脓毒症发生后,氧化磷酸化转变成有氧糖酵解[32]。在巨噬细胞脓毒症模型中,有氧糖酵解增强,促使巨噬细胞向依赖有氧糖酵解供能的M1型转化而不是氧化磷酸化供能的M2型巨噬细胞[33]。有研究发现,小鼠脓毒症ALI的发生与缺氧诱导因子-1α (hypoxia inducible factor-1α, HIF-1α)、丙酮酸激酶M2 (pyruvate kinase isozyme type M2, PKM2)和LDHA等有氧糖酵解指标的表达增加及炎症因子IL-1β、白细胞介素-6 (IL-6)和肿瘤坏死因子-α (tumor necrosis factor-α,TNF-α)的释放增加有关[34]。XU等[12]研究证实,有氧糖酵解关键酶6-磷酸果糖-2-激酶/果糖-2,6-二磷酸酶3 (6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3, PFKFB3)可促进有氧糖酵解,增加炎症因子IL-1β、IL-6的释放及其mRNA的表达水平,加重小鼠脓毒症ALI,这些研究结果与本实验结果一致。

3.2 小鼠SA-AKI的减轻可能与PF降低有氧糖酵解水平和炎症反应有关

血必净临床应用于脓毒症患者治疗,疗效显著,但尚不明确哪些成分,通过何种机制发挥治疗作用[35]。PF作为血必净的主要成分之一,在细胞实验中有良好的抗炎作用[36]。本研究观察到,小鼠腹腔注射PF后,LPS+PF组小鼠肾组织Paller评分下降,肾损伤标志物Scr、NGAL降低,肾损伤得到减轻。同时,小鼠肾组织HK2、LDHA和血清乳酸等有氧糖酵解指标及炎症因子IL-1β、IL-18含量减少。因此,推测小鼠SA-AKI的减轻可能与PF降低有氧糖酵解水平和炎症反应有关。有氧糖酵解的调节已成为脓毒症干预的潜在策略。研究发现,PF可以下调巨噬细胞中PKM2、LDHA的mRNA和蛋白表达水平,降低有氧糖酵解水平[37]。Cynaroside可以抑制HK2、PFKFB3和HIF-1α等有氧糖酵解关键蛋白及酶的表达,促进巨噬细胞M1型向M2型转变[10]。小鼠脓毒症多器官功能损伤的缓解与辣椒素降低PKM2、LDHA等有氧糖酵解关键酶的表达,减少IL-1β、IL-6和TNF-α等炎症因子的释放有

关[38]，这与本研究结果相似。后续将在mRNA水平进行相关指标的检测，以弥补本研究的局限性。

3.3 PF可能通过抑制β-catenin/c-Myc通路，降低肾组织有氧糖酵解水平及炎症反应，缓解小鼠SA-AKI

为阐明PF的保护机制，本研究在PF治疗的基础上给予小鼠腹腔注射β-catenin特异性激动剂BML284，BML284可靶向激活β-catenin，提高β-catenin/c-Myc通路活性[21]。结果发现，LPS+PF+BML284组小鼠肾组织Paller评分上升，肾损伤标志物Scr、NGAL增加，肾损伤反而加重；同时肾组织HK2、LDHA和血清乳酸等有氧糖酵解指标及炎症因子IL-1β、IL-18含量增加；通路蛋白总β-catenin、p-β-catenin^{Y654}、细胞核β-catenin以及c-Myc表达升高。因此，小鼠SA-AKI的加重可能与BML284激活β-catenin/c-Myc通路，增加有氧糖酵解及炎症反应有关。ZHOU等[39]发现，在脓毒症巨噬细胞模型中，与LPS组相比，LPS+BML284组β-catenin的表达水平上升，炎症因子IL-1β、IL-6、TNF-α的释放增加。动脉粥样硬化细胞模型中，miR-100-5p可以降低IL-1β、IL-6和TNF-α的含量，但经BML284干预后上述炎症因子反而增加，炎症反应加重，动脉粥样硬化的进展明显加快[15]。

急性肾损伤（acute kidney injury，AKI）发生后，β-catenin/c-Myc通路被激活，p-β-catenin^{Y654}增加，细胞质中总β-catenin增加并发生核易位，增加细胞核β-catenin的表达，从而促进下游c-Myc的转录和翻译[40-42]。HU等[43]研究证明，AKI的动物及细胞模型中，盐诱导激酶1抑制p-β-catenin^{Y654}、细胞核β-catenin的表达，减少IL-1β的mRNA水平，缓解AKI。SA-AKI的动物和细胞模型中，Fractalkine可增加总β-catenin、c-Myc的表达水平，促进炎症反应，加重SA-AKI[44]。有研究证实，c-Myc与HK2、LDHA基因上游的E-box（5′-CACGTG-3′）结合，促进HK2、LDHA转录及翻译，增加有氧糖酵解速率[45]。研究表明，小鼠非小细胞肺癌的增殖与β-catenin/c-Myc通路激活、LDHA的表达增加和有氧糖酵解水平的增强有关[46]。上述研究结果与本实验结果一致。

本研究观察到LPS+PF+BML284组与LPS+PF组相反的结果，表明BML284可以逆转PF的保护作用，推测PF可能通过抑制β-catenin/c-Myc通路，降低肾组织有氧糖酵解水平及炎症反应，缓解小鼠SA-AKI。仅在动物实验观察到上述结果，尚需在细胞实验进一步验证。

综上所述，PF可以减轻SA-AKI，其机制可能是通过抑制β-catenin/c-Myc通路，降低肾组织有氧糖酵解水平，减轻炎症反应。这为PF治疗SA-AKI提供了理论依据。

利益冲突 本文所有作者声明不存在利益冲突

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