论著·基础医学

腺苷二醛调控甲基化代谢及H3K4me3修饰抑制NF-κB通路减轻脓毒症炎症与组织损伤

赵雨婷[1,2],刘 鑫[3],何宇龙[1,2],王宇洁[1,2],潘 雪[1,2],邵欣宇[1,2],王 棚[1,2],罗昕玥[1,2],李小丽[1,2],邱红梅[1,2]
[1]重庆医科大学药学院药理学系,重庆;[2]重庆高校生物化学与分子药理学重点实验室,重庆;[3]陆军军医大学(第三军医大学)第一附属医院临床医学研究中心,重庆

[摘要] **目的** 探讨腺苷二醛(adenosine dialdehyde,Adox)对脓毒症诱导的炎症反应及组织损伤的影响,并分析其对甲基化代谢稳态、H3K4me3修饰及NF-κB信号通路的调控作用。**方法** 细胞实验:① CCK-8法检测Adox(2.5~40 μmol/L)处理小鼠单核巨噬细胞白血病细胞(RAW264.7)和人单核细胞白血病细胞系(THP-1)24 h后对细胞活力的影响,以筛选Adox的安全剂量范围。②ELISA检测不同浓度Adox(2.5、5、10、20、40 μmol/L)对脂多糖(lipopolysaccharide,LPS)诱导的炎症因子释放的影响,并检测10 μmol/L Adox对100 ng/mL LPS不同刺激时间中巨噬细胞炎症因子释放的影响。③将RAW264.7和THP-1细胞分为:Medium组、LPS100组(100 ng/mL LPS刺激4 h)及LPS100+Adox组(10 μmol/L Adox和LPS共处理4 h),ELISA检测细胞上清TNF-α、IL-6、SAM及SAH水平;Western blot检测H3K4me3、SAHH及NF-κB/IκBα信号通路相关蛋白的表达;RT-qPCR检测TNF-α、IL-6 mRNA表达。动物实验:采用6~8周龄雄性C57BL/6J小鼠(体质量18~22 g),随机分为(n=6):Sham(假手术)组、CLP[盲肠结扎穿孔(cecal ligation and puncture,CLP)脓毒症模型]组;CLP+Adox组(术前4 h腹腔注射2 mg/kg Adox后建立CLP模型),术后6 h取材。ELISA检测小鼠血清TNF-α、IL-6水平;HE染色及病理评分(0~12分)评价肺、脾组织损伤;Western blot检测肺、脾组织中H3K4me3、SAHH及NF-κB/IκBα信号通路相关蛋白的表达。**结果** 细胞实验:CCK-8结果显示,Adox在2.5~40 μmol/L范围内对RAW264.7和THP-1细胞无明显毒性。ELISA结果表明,Adox可呈浓度依赖性和时间依赖性显著降低细胞中TNF-α和IL-6的释放水平(P<0.01),10 μmol/L Adox在显著抑制炎症因子的同时对细胞活力无明显影响,用于后续实验。Adox处理后RAW264.7细胞上清中SAM水平降低,SAH水平升高,SAM/SAH比值下降(P<0.01)。Western blot结果显示,Adox可显著降低炎症状态下RAW264.7和THP-1细胞中SAHH及H3K4me3蛋白表达水平(P<0.01)并抑制NF-κB信号通路活化。动物实验:ELISA结果显示,Adox可显著降低CLP诱导的脓毒症小鼠血清TNF-α和IL-6水平(P<0.01)。HE染色及病理评分显示,Adox可改善脓毒症小鼠肺、脾组织损伤。Western blot结果显示,Adox可显著降低CLP小鼠肺、脾组织中H3K4me3水平,并抑制NF-κB/IκBα信号通路活化。**结论** Adox可减轻脓毒症相关炎症反应及组织损伤,其机制可能与调控SAHH介导的SAM/SAH代谢、降低H3K4me3修饰水平以及抑制NF-κB信号通路活化有关。上述结果提示,靶向甲基化代谢及组蛋白甲基化修饰可能为脓毒症炎症干预提供新的治疗策略。

[关键词] 脓毒症;炎症反应;组蛋白H3K4me3;腺苷二醛;核因子-κB
[中图法分类号] R342;R394.3;R631 [文献标志码] A

[基金项目] 重庆市自然科学基金面上项目(CSTB2025NSCQ-GPX1241)

[通信作者] 李小丽,E-mail:lixiaoli@cqmu.edu.cn
邱红梅,E-mail:qiuhongmei@cqmu.edu.cn

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引用格式:赵雨婷,刘鑫,何宇龙,等.腺苷二醛调控甲基化代谢及H3K4me3修饰抑制NF-κB通路减轻脓毒症炎症与组织损伤[J].陆军军医大学学报,2026,48(18):2665-2676.DOI:10.16016/j.2097-0927.202606028.

Citation:Zhao Y T,Liu X,He Y L,*et al*. Adenosine dialdehyde regulates methylation metabolism and H3K4me3 modification to inhibit the NF-κB pathway and attenuate sepsis-induced inflammation and tissue injury[J]. J Army Med Univ,2026,48(18):2665-2676. DOI:10.16016/j.2097-0927.202606028.

Adenosine dialdehyde regulates methylation metabolism and H3K4me3 modification to inhibit the NF-κB pathway and attenuate sepsis-induced inflammation and tissue injury

Zhao Yuting[1,2], Liu Xin[3], He Yulong[1,2], Wang Yujie[1,2], Pan Xue[1,2], Shao Xinyu[1,2], Wang Peng[1,2], Luo Xinyue[1,2], Li Xiaoli[1,2], Qiu Hongmei[1,2] [1]Department of Pharmacology, College of Pharmacy, Chongqing Medical University, Chongqing; [2]Key Laboratory of Biochemistry and Molecular Pharmacology of Higher Education in Chongqing, Chongqing; [3]Clinical Research Center, First Affiliated Hospital, Army Military Medical University (Third Military Medical University), Chongqing, China

[**Abstract**] Objective To investigate the effects of adenosine dialdehyde (Adox) on sepsis-induced inflammatory response and tissue injury, and to analyze its regulatory roles in methylation metabolic homeostasis, H3K4me3 modification, and the NF-κB signaling pathway. Methods *In vitro* experiments: ① Mouse mononuclear macrophage leukemia cells (RAW264.7) and human monocytic leukemia cells (THP-1) were subjected. CCK-8 assay was performed to determine the effects of Adox (2.5–40 μmol/L) on cell viability after 24 h of treatment in RAW264.7 and THP-1 cells to screen the safe dose range of Adox. ② ELISA was conducted to assess the impact of various Adox concentrations (2.5, 5, 10, 20, 40 μmol/L) on lipopolysaccharide (LPS)-induced inflammatory cytokine release, and to examine the effect of 10 μmol/L Adox on the release of inflammatory cytokines at different time points following 100 ng/mL LPS stimulation. ③ RAW264.7 and THP-1 cells were divided into a Medium group, a LPS100 group (stimulated with 100 ng/mL LPS for 4 h), and a LPS100+Adox group (co-treated with 10 μmol/L Adox and LPS for 4 h). ELISA was employed to measure TNF-α, IL-6, SAM and SAH levels in cell supernatants; Western blot was utilized to detect the protein expression of H3K4me3, SAHH and NF-κB/IκBα signaling pathway-related molecules; RT-qPCR was carried out to measure the mRNA expression of TNF-α and IL-6. Animal experiments: Male C57BL/6J mice (6 to 8 weeks old, weighing 18 to 22 g) were randomly assigned to a Sham group, a cecal ligation and puncture group (CLP, as a sepsis model), and a CLP+Adox group (intraperitoneal injection of 2 mg/kg Adox administered 4 h prior to CLP surgery). Samples were collected 6 h after modeling. ELISA was used to detect serum TNF-α and IL-6 levels in mice; HE staining and pathological scoring (0–12) were utilized to evaluate lung and spleen tissue injury; Western blot was performed to detect the expression of H3K4me3, SAHH, and NF-κB/IκBα signaling pathway-related proteins in lung and spleen tissues. Results *In vitro* experiments: CCK-8 results demonstrated that Adox had no obvious toxicity to RAW264.7 and THP-1 cells within the range of 2.5 to 40 μmol/L. ELISA revealed that Adox significantly reduced the released levels of TNF-α and IL-6 in a concentration- and time-dependent manner ($P<0.01$). Notably, 10 μmol/L Adox significantly suppressed inflammatory cytokines without affecting cell viability, and was therefore selected for subsequent experiments. Furthermore, Adox treatment decreased SAM levels, increased SAH levels, and reduced the SAM/SAH ratio in RAW264.7 cell supernatants ($P<0.01$). Western blot analysis demonstrated that Adox significantly downregulated the protein expression of SAHH and H3K4me3 in RAW264.7 and THP-1 cells under inflammatory conditions ($P<0.01$), and inhibited the activation of the NF-κB signaling pathway. *In vivo* experiments: ELISA results showed that Adox significantly decreased serum TNF-α and IL-6 levels in CLP-induced septic mice ($P<0.01$). HE staining and pathological scoring demonstrated that Adox ameliorated lung and spleen injuries in septic mice. Western blot analysis indicated that Adox significantly reduced H3K4me3 levels and suppressed NF-κB/IκBα signaling pathway activation in the lung and spleen tissues of CLP mice. Conclusion Adox alleviates sepsis-related inflammatory response and tissue injury, and its mechanism may be related to the regulation of SAHH-mediated SAM/SAH metabolism, reduction of H3K4me3

modification levels, and inhibition of NF-κB signaling pathway activation. These findings suggest that targeting methylation metabolism and histone methylation modifications may provide a novel therapeutic strategy for intervening in sepsis-associated inflammation.

[**Key words**] sepsis; inflammatory response; histone H3K4me3; adenosine dialdehyde; nuclear factor-κB

Supported by the General Project of Natural Science Foundation of Chongqing (CSTB2025NSCQ-GPX1241). Corresponding authors: Li Xiaoli, E-mail: lixiaoli@cqmu.edu.cn; Qiu Hongmei, E-mail: qiuhongmei@cqmu.edu.cn

脓毒症(Sepsis)是一种由宿主对感染反应失调引发的危及生命的器官功能障碍[1],具有起病急、发生快、致死率高等特点,是重症医学领域的重要临床难题[2]。脓毒症的发生发展与机体过度炎症反应密切相关,脂多糖(lipopolysaccharide,LPS)等病原相关分子可使先天免疫核心效应细胞如巨噬细胞激活,促使肿瘤坏死因子-α(TNF-α)、白细胞介素-6(IL-6)等促炎因子大量释放[3],过度的炎症反应导致组织损伤和多器官功能障碍[4]。在这一过程中,核因子-κB(nuclear factor-κB,NF-κB)信号通路是调控炎症反应的核心通路之一,其异常激活可促进多种促炎因子如TNF-α、IL-6的释放以及炎症相关基因转录,加剧脓毒症的炎症损伤效应[5-6]。因此,寻找能够有效调控NF-κB信号通路及炎症反应的新型干预策略,对脓毒症治疗具有重要意义[7-8]。

近年来,组蛋白修饰作为表观遗传调控的核心机制之一,通过重塑染色质结构、调控基因转录活性,在炎症相关性疾病中的作用日益受到关注[9]。组蛋白H3第4位赖氨酸三甲基化(histone H3 lysine 4 trimethylation,H3K4me3),通常富集于活跃转录基因的启动子区域,与基因转录激活密切相关[10]。研究发现,H3K4me3不仅参与胚胎发育和细胞分化,也参与免疫细胞活化、炎症基因转录以及内毒素耐受等过程[11-13]。研究表明,NF-κB信号通路与H3K4me3修饰之间存在双向调控关系。一方面,NF-κB转录活化亚基p65可招募组蛋白甲基转移酶至靶基因启动子,促进H3K4me3沉积,从而增强炎症相关基因转录;另一方面,H3K4me3维持开放染色质状态,促进NF-κB与DNA结合并增强其转录活性[11-13]。这种NF-κB与H3K4me3的双向调控关系,为阐明脓毒症炎症反应的表观遗传调控机制提供了新的视角。

组蛋白的甲基化水平不仅受到甲基转移酶和去甲基化酶的调控,也与细胞内甲基化代谢状态密切相关。S-腺苷甲硫氨酸(s-adenosylmethionine,SAM)是细胞内主要甲基供体,可为组蛋白及DNA等底物的甲基化修饰提供甲基;其代谢产物S-腺苷同型半胱氨酸(s-adenosyl-L-homocysteine,SAH)则可反馈性抑制甲基转移酶活性,从而限制甲基化反应的进行。S-腺苷同型半胱氨酸水解酶(s-adenosylhomocysteine hydrolase,SAHH/AHCY)通过催化SAH水解,降低细胞内SAH蓄积,维持SAM/SAH平衡及细胞甲基化稳态[14]。因此,调控SAHH活性及SAM/SAH代谢稳态可能进一步影响H3K4me3等组蛋白甲基化修饰,从而调控炎症相关基因转录。

腺苷二醛(adenosine dialdehyde,Adox)是一种经典的SAHH抑制剂,可通过抑制SAHH活性导致细胞内SAH蓄积,进而抑制甲基转移酶活性,影响组蛋白甲基化等表观遗传修饰过程[15]。近年来研究发现,Adox除调控甲基化代谢外,还具有抗炎作用。研究表明,Adox能够抑制Tax介导的NF-κB信号通路激活,促进IκBα稳定,从而阻断NF-κB的转录过程[16]。此外,另一种SAHH抑制剂3-Deazaadenosine可通过直接抑制MEK1/2和IKKα/β激酶活性,阻断AP-1和NF-κB信号通路发挥抗炎作用[17]。上述研究提示,Adox不仅能够干预SAM/SAH代谢平衡并调控表观遗传修饰,还可能通过抑制NF-κB等炎症相关信号通路发挥抗炎作用。然而,在脓毒症炎症状态下,Adox是否能够通过调控甲基化代谢及组蛋白修饰减轻炎症损伤,其具体作用机制尚不明确。因此,本研究采用LPS诱导巨噬细胞炎症模型及CLP诱导脓毒症小鼠模型,系统评价Adox对炎症反应及组织损伤的影响,并重点探讨其对SAHH-SAM/SAH代谢,H3K4me3修饰及NF-κB信号通路的调控作用,以阐明其减轻脓毒症炎症损伤的可能机制。

1 材料与方法

1.1 实验动物

SPF级雄性C57BL/6J小鼠36只,6~8周龄,体质量为18~22 g,饲养于SPF级实验动物房内。本研究方案经陆军军医大学实验动物福利伦理审查委员会批准(AMUWEC20210998),所有动物实验均符合实验动物伦理及福利要求。

1.2 实验细胞

小鼠单核巨噬细胞RAW264.7购自普诺赛生命科技有限公司,于实验室液氮冻存保种。细胞常规培养于含10%胎牛血清的完全培养基中,于37 ℃、5% CO_2细胞孵箱中培养,待细胞长满至80%左右时进行传代培养。人急性单核细胞白血病细胞THP-1购自普诺赛生命科技有限公司,培养于含10%胎牛血清的RPMI Medium 1640完全培养基中,于37 ℃、5% CO_2细胞孵箱中培养,待细胞密度达到(5~8)×10^5/mL时进行传代培养。所有细胞系均通过STR分析进行鉴定,同时支原体检测结果均为阴性。

1.3 主要试剂

LPS购自美国Sigma公司;胎牛血清购自武汉普诺赛生命科技有限公司;DMEM高糖培养基购自赛默飞世尔科技公司;腺苷二醛(adenosine dialdehyde,Adox)购自美国Selleck公司;兔抗H3K4me3,p-IκBα重组抗体购自美国CST公司;兔抗NF-κB,p-NF-κB,IκBα多克隆抗体购自武汉三鹰生物有限技术公司;SAHH重组兔单克隆抗体购自华安生物;鼠抗β-actin多克隆抗体购自美国SantaCruz公司;HRP-conjugated Goat anti-Rabbit IgG(H+L)和HRP-conjugated Goat anti-Mouse IgG(H+L)购自ABclonal公司;Western及IP细胞裂解液、RIPA裂解液、蛋白酶抑制剂及细胞核与细胞浆蛋白抽提试剂盒等购自碧云天生物技术有限公司;ECL试剂盒购自Advansta公司;S-腺苷甲硫氨酸(s-adenosylmethionine,SAM)及S-腺苷-L-同型半胱氨酸(s-adenosyl-l-homocysteine,SAH)ELISA试剂盒购自江苏酶免公司。

1.4 细胞实验步骤

1.4.1 内毒素刺激的炎症细胞模型的建立 细胞分为正常组(Medium组)、模型组(LPS100组),每组设置5个复孔。Medium组加入DMEM培养基;LPS100组加入含100 ng/mL LPS的DMEM培养基处理4 h[18]。

1.4.2 CCK-8检测RAW264.7和THP-1细胞活力 RAW264.7和THP-1细胞分别接种于96孔板,待细胞贴壁后,加入2.5、5、10、20、40 μmol/L Adox处理24 h后,加入100 μL含10% CCK-8试剂的DMEM培养基,于37 ℃细胞培养箱孵育1 h后,使用酶标仪于450 nm波长处检测光密度D(450)值。

1.4.3 ELISA检测Adox对LPS诱导的RAW264.7细胞中炎症因子、SAM及SAH含量的影响 RAW264.7接种于96孔板,待细胞贴壁后,进行处理:①Adox对100 ng/mL LPS处理4 h后RAW264.7细胞不同时间点后炎症因子释放水平的影响:加入10 μmol/L Adox预处理30 min后,再加入100 ng/mL LPS分别刺激3、6、12 h后,收集细胞上清液,用ELISA法检测TNF-α和IL-6水平。②不同浓度Adox对LPS刺激RAW264.7细胞后炎症因子释放水平的影响:加入2.5、5、10、20、40 μmol/L Adox预处理RAW264.7细胞30 min后,再加入100 ng/mL LPS刺激4 h后,收集细胞上清液,用ELISA法检测TNF-α和IL-6水平。③Adox对LPS诱导的RAW264.7细胞上清中SAM和SAH含量的影响:加入10 μmol/L Adox预处理30 min后,再加入100 ng/mL LPS分别刺激4 h后,收集细胞上清液,用ELISA法检测SAM和SAH水平。

1.4.4 RT-qPCR法检测LPS刺激后细胞中TNF-α和IL-6的mRNA表达水平 收集细胞,用RNAiso Plus试剂提取RNA,参照反转录反应试剂说明书合成cDNA。RT-qPCR反应体系配制后,在荧光定量PCR仪中行Real time PCR反应。按照以下程序进行扩增:预变性95 ℃ 30 s,PCR反应95 ℃ 5 s,60 ℃ 30 s 39个循环。采用$2^{-\Delta\Delta Ct}$法分析目的基因相对表达量,数据统计以Ct值为基准,实验全程设置了4次重复。实验中使用的引物见表1。

表1 实时荧光定量PCR的引物序列

Table 1 Primer sequences for real-time quantitative PCR

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
TNF-α	GCAAAGGGAGAGTGGTCA	CTGGCTCTGTGAGGAAGG
IL-6	AGGCATAACGCACTAGGTTT	ACAGAAGGAGTGGCTAAGGA
β-actin	GTGCTATGCTCTAGACG	ATGCCACAGGATTCCATACC

1.4.5 Western blot法检测Adox对LPS诱导的RAW264.7细胞中H3K4me3、SAHH和NF-κB信号通路相关蛋白表达水平的影响 RAW264.7和THP-1细胞均接种于10 cm培养皿中,正常组仅用无血清DMEM培养基处理;模型组加入100 ng/mL LPS处理4 h;给药组:加入Adox(10 μmol/L)处理30 min后,再加入100 ng/mL LPS共同处理4 h。收集细胞,采用含10%蛋白酶抑制剂的Western及IP细胞裂解液提取细胞总蛋白,采用细胞核蛋白抽取试剂盒提取细胞核蛋白(严格按照试剂说明书进行提取操作),BCA法测定蛋白浓度,调整蛋白上样量后,100 ℃煮沸10 min。取30 μg蛋白进行SDS-PAGE电泳。电泳结束后将蛋白转移至PVDF膜,封闭液室温封闭1 h。加入稀释后的一抗在4 ℃摇床孵育过夜。次日,TBST洗涤5次,再在室温孵育二抗,TBST洗5次后,在Tanon 4600成像系统中使用ECL底物通过增强化学发光显现目标蛋白质条带。

1.5 动物实验步骤

1.5.1 盲肠结扎穿孔术(cecal ligation and puncture,CLP)脓毒症小鼠模型的建立 小鼠随机分为(n=6):①Sham(假手术)组:术前腹腔注射等体积生理盐水,开腹分离出盲肠后回纳腹腔,缝合关腹。②CLP组:术前腹腔注射等体积生理盐水,开腹分离出盲肠结扎1.0 cm,采用12号钢针穿孔后,挤出少量粪便,缝合关腹。③CLP组+Adox组:术前腹腔注射Adox(2 mg/kg),4 h后步骤同上行CLP(记为术后0 h)。3组小鼠均于术后6 h进行取材[18-19]。

1.5.2 Western blot法检测小鼠肺、脾组织中H3K4me3、SAHH和NF-κB信号通路相关蛋白表达水平 将小鼠肺、脾组织放入在液氮中冻硬的研钵中研磨得到组织匀浆,采用含10%蛋白酶抑制剂的RIPA裂解液提取组织蛋白,其余步骤同前。

1.5.3 HE染色 将固定后的肺、脾组织经脱水、透明、浸蜡、包埋处理后,制作厚度为4~6 μm的连续组织切片,再经过脱蜡、水化后用苏木精进行染色,通过光学显微镜观察、采集图像并进行病理损伤评分[22-23]。

1.6 统计学分析

使用GraphPad Prism 9.0软件进行统计学作图,使用ImageJ 1.8.0软件分析蛋白表达定量。每组实验至少重复3次,所有数值结果均以$\bar{x}\pm s$表示,多组间比较采用单因素方差分析,P<0.05表示差异具有统计学意义。

2 结果

2.1 Adox抑制LPS诱导的RAW264.7细胞中炎症因子TNF-α和IL-6的释放

CCK-8法评估Adox的细胞毒性结果显示:Adox在2.5~40 μmol/L浓度范围内对RAW264.7和THP-1细胞活力均无明显影响,细胞存活率均高于80%,结果提示在此浓度区间Adox对2株细胞无明显的毒性作用(图1A、B)。

ELISA结果表明:2.5、5、10、20、40 μmol/L Adox预处理RAW264.7细胞30 min后,再加入100 ng/mL LPS刺激4 h,TNF-α和IL-6的释放水平与单独的LPS100组相比,呈浓度依赖性显著降低(P<0.01,图1C、D)。100 ng/mL LPS刺激可呈时间依赖性地促进RAW264.7细胞中TNF-α和IL-6的释放(P<0.01,图1E、F);与LPS100刺激的相应时间点相比,10 μmol/L Adox可显著抑制炎症因子TNF-α和IL-6的释放(P<0.01,图1E、F)。10 μmol/L Adox在显著抑制炎症因子的同时对细胞活力无显著影响,因此选用该浓度为后续的细胞实验浓度。上述结果提示Adox具有抑制LPS诱导巨噬细胞炎症反应的作用。

2.2 Adox抑制LPS诱导的RAW264.7细胞中TNF-α和IL-6 mRNA的表达

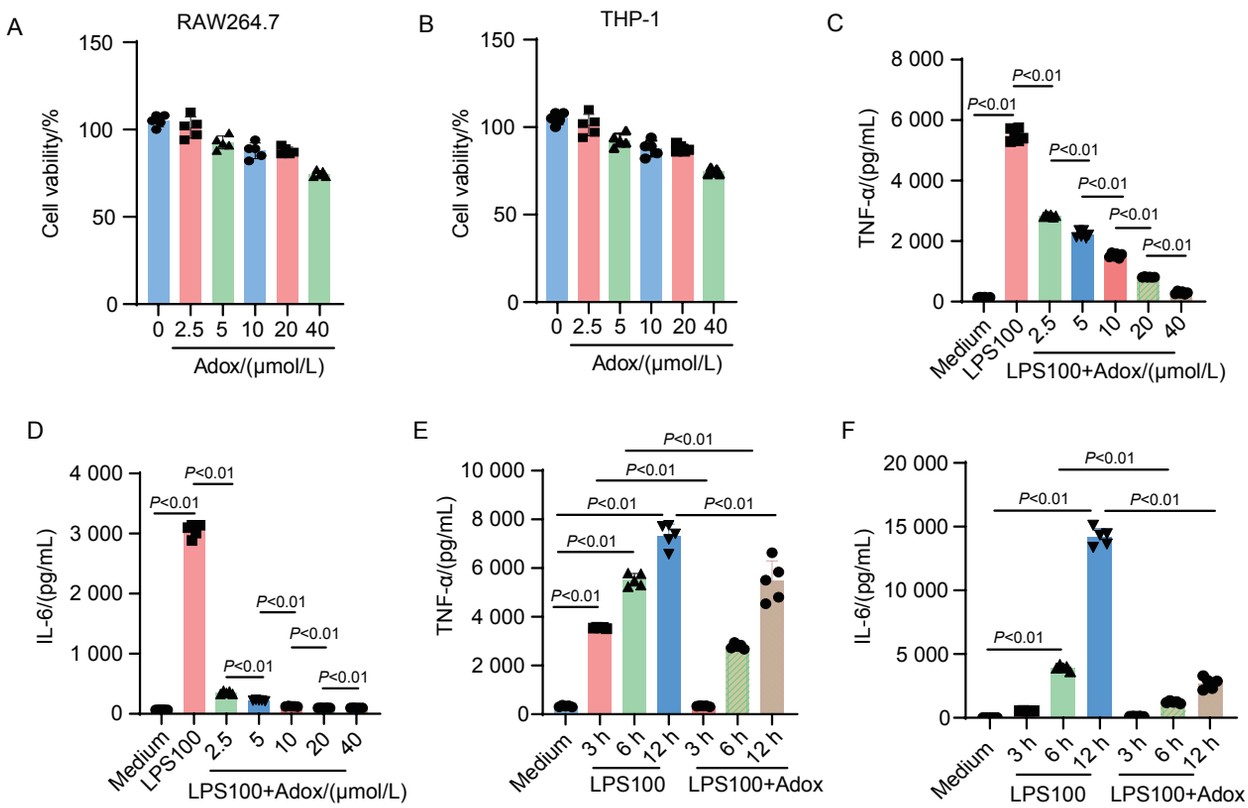
RT-qPCR结果显示,与Medium组相比,在100 ng/mL LPS刺激下RAW264.7细胞中TNF-α和IL-6 mRNA表达显著升高(P<0.01);Adox处理后可明显降低TNF-α和IL-6 mRNA表达(P<0.01,图2A~B)。结果提示,Adox可在转录水平抑制炎症相关基因的表达。

2.3 Adox减轻CLP诱导的小鼠全身炎症反应及组织损伤

为验证Adox在体内的抗炎作用,建立CLP诱导的脓毒症小鼠模型。ELISA检测结果显示,CLP术后6 h小鼠血清中的TNF-α和IL-6水平较Sham组显著升高(P<0.01,图3A);CLP+Adox组可明显降低血清中炎症因子的水平(P<0.01,图3B)。为进一步评估组织损伤情况,对小鼠的肺和脾组织进行了HE染色和病理评分。结果表明,Sham组肺实质结构正常,肺泡结构完整,无明显病理病变;CLP组肺泡结构严重破坏,肺泡壁增厚,伴有明显的肺泡内出血及间质水肿;CLP+Adox组肺组织病理损伤显著减轻,肺泡壁增厚程度减轻,炎症细胞浸润及肺泡腔内出血及间质水肿明显减轻(图3C)。同时,Sham组小鼠脾组织结构清晰完整,白髓与红髓分界清晰,白髓区域内脾小结结构完整,未见明显的充血、出血及炎症细胞浸润;CLP组小鼠白髓结构破坏,红髓区域显著扩展,白髓出现大量炎性细胞浸润,红髓、白髓分界不清;CLP+Adox组白髓、红髓结构较CLP组明显改善;红髓区域充血和出血程度显著减轻,炎性细胞浸润明显减少,细胞排列趋于规则(图3D)。以上结果表明,Adox能够减轻CLP诱导的肺和脾组织的损伤。

2.4 脓毒症炎症状态下H3K4me3表达上调

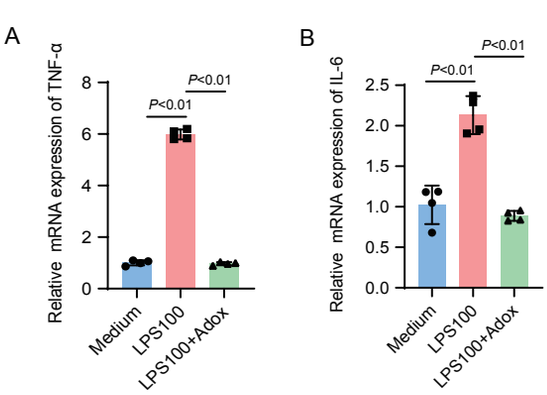
Western blot结果显示,Medium组中RAW264.7和THP-1细胞中H3K4me3表达均较低;与Medium组相比,LPS100组中H3K4me3的表达均显著升高(P<0.01,图4A~B)。在体内实验中,Sham组小鼠肺和脾组织中H3K4me3蛋白表达均较低,与Sham组相比,CLP组中H3K4me3蛋白表达显著增加(P<0.01,图4C~D)。上述结果表明,H3K4me3在炎症状态下表达显著上调。



A and B: Cell viability of RAW264.7 (A) and THP-1 (B) cells after treatment with 2.5 to 40 μmol/L Adox for 24 h measured by CCK-8 assay; C and D: Secretion levels of TNF-α (C) and IL-6 (D) in RAW264.7 cells across groups (pre-treatment with 2.5, 5, 10, 20 and 40 μmol/L Adox for 30 min, followed by stimulation with 100 ng/mL LPS for 4 h) determined by ELISA; E and F: Concentrations of TNF-α (E) and IL-6 (F) in RAW264.7 cells across groups (pre-incubated with 10 μmol/L Adox for 30 min and then stimulated by 100 ng/mL LPS for 3, 6 and 12 h) detected by ELISA

图1 CCK-8、ELISA分别检测RAW264.7和THP-1细胞活力及炎症因子释放的影响 (n=5, $\bar{x}\pm s$)

Figure 1 Effects of different concentrations of Adox on cell viability and inflammatory factor release in RAW264.7 and THP-1 cells by CCK-8 assay and ELISA (n=5, $\bar{x}\pm s$)



A and B: mRNA expression levels of TNF-α (A) and IL-6 (B) in different groups of RAW264.7 cells by RT-qPCR

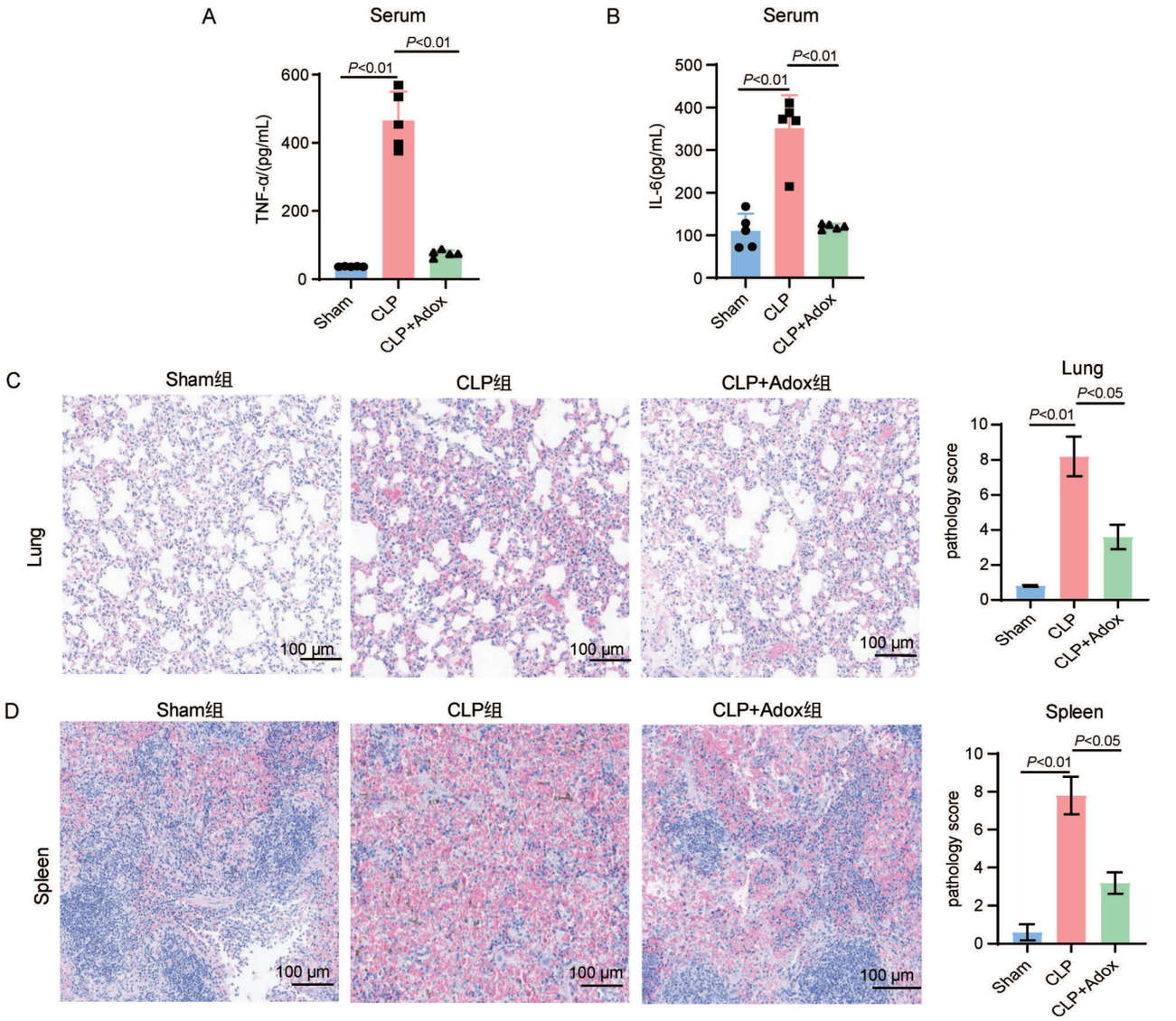
图2 RT-qPCR检测RAW264.7细胞中TNF-α和IL-6的mRNA表达水平 (n=4, $\bar{x}\pm s$)

Figure 2 mRNA expression levels of TNF-α and IL-6 in RAW264.7 cells detected by RT-qPCR (n=4, $\bar{x}\pm s$)

2.5 Adox通过调控SAHH及甲基化代谢稳态降低H3K4me3水平

为探讨Adox调控H3K4me3修饰的代谢基础,检测细胞中SAHH蛋白表达水平、细胞上清中SAM和SAH的代谢变化,以及H3K4me3蛋白表达水平。Western blot结果显示,与Medium组相比,100 ng/mL LPS刺激后RAW264.7细胞和THP-1细胞中SAHH蛋白表达均显著升高(P<0.01);经Adox干预后,两种细胞中SAHH蛋白表达均明显下降(P<0.01,图5A~B)。

进一步采用ELISA检测SAM和SAH水平。结果显示,与Medium组相比,LPS100组细胞上清中SAM含量显著升高(P<0.01);Adox干预后,SAM水平明显下降(P<0.01,图5C)。与此同时,LPS100组SAH水平无明显变化,而Adox处理后SAH水平显著升高(P<0.01,图5D)。由于SAM/SAH比值常被用作反映细胞



A and B: Serum levels of TNF-α (A) and IL-6 (B) in mice determined by ELISA (n=5, $\bar{x}\pm s$); C and D: Pathological changes in the lung (C) and spleen (D) tissues by HE staining and pathological scores (n=3, $\bar{x}\pm s$)

图3 ELISA检测小鼠血清炎症因子水平、HE染色检测小鼠肺、脾组织病理损伤情况

Figure 3 Serum inflammatory factor levels by ELISA and pathological injury of the lung and spleen tissues by HE staining

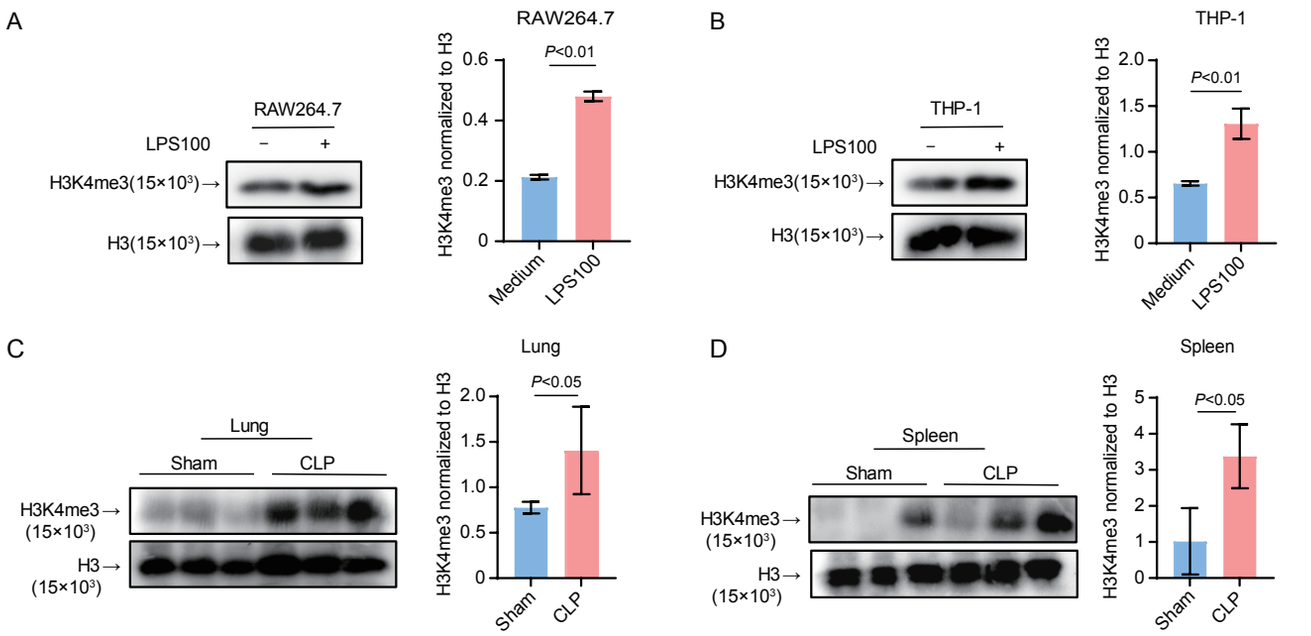
甲基化潜能的重要指标,进一步分析发现,LPS100组SAM/SAH比值较Medium组显著升高(P<0.01,图5E),提示炎症状态下细胞甲基化潜能增强;Adox干预后,该比值显著下降(P<0.01,图5E),表明其能够降低细胞甲基化潜能并抑制甲基化代谢通量。

Western blot结果进一步显示,LPS刺激后RAW264.7和THP-1细胞中H3K4me3表达显著上调(P<0.01);Adox处理后,两种细胞中H3K4me3水平均显著降低(P<0.01,图5F~G)。体内实验中,CLP小鼠肺和脾组织中H3K4me3表达较Sham组显著升高(P<0.01),Adox干预后显著降低(P<0.01,图5H~I)。

综合上述结果,Adox可下调SAHH蛋白表达,促进SAH蓄积并降低SAM/SAH比值,从而干预细胞甲基化代谢稳态,并进一步降低H3K4me3修饰水平,为其调控炎症状态下表观遗传修饰提供了代谢学依据。

2.6 Adox抑制脓毒症炎症状态下NF-κB信号通路的激活

为进一步明确Adox对NF-κB信号通路的影响,我们检测了炎症状态下NF-κB通路相关蛋白的表达。



A and B: Protein expression of H3K4me3 in RAW264.7 (A) and THP-1 (B) cells in the Medium group (DMEM) and the LPS100 group; C and D: H3K4me3 protein expression in the lung (C) and spleen (D) tissues of CLP mice by Western blot

图4 Western blot检测炎症状态下H3K4me3蛋白表达水平(n=3,$\bar{x}\pm s$)

Figure 4 H3K4me3 protein expression levels under inflammatory conditions by Western blot (n=3,$\bar{x}\pm s$)

Western blot结果显示,LPS刺激可显著升高RAW264.7和THP-1细胞中p-NF-κB和p-IκBα的水平(P<0.01);Adox处理后可明显降低p-NF-κB和p-IκBα的蛋白表达水平(P<0.01,图6A~B)。体内小鼠肺、脾组织中的结果与体外一致(图6C~D)。提示Adox可能通过抑制IκBα磷酸化及NF-κB信号通路活化发挥抗炎作用。

3 讨论

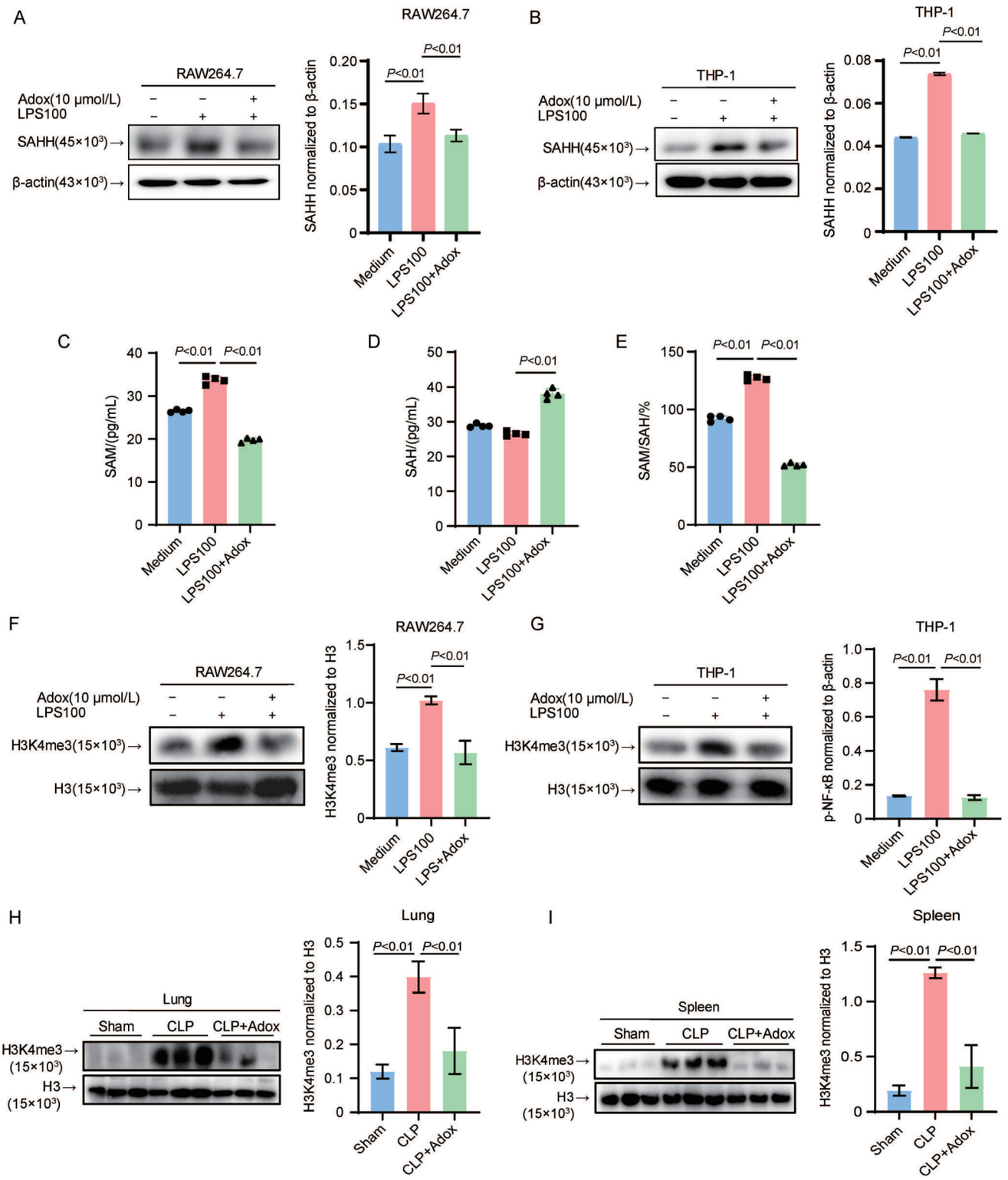
脓毒症是一种由宿主对感染反应失调引发的危及生命的器官功能障碍,以失控的炎症反应和进行性器官功能损伤为主要病理特征[1]。巨噬细胞作为先天免疫反应的重要效应细胞,在脓毒症早期被病原体相关分子模式激活后,可大量释放TNF-α、IL-6等促炎细胞因子,诱发全身炎症反应并导致组织损伤[20]。本研究采用LPS诱导的炎症巨噬细胞模型和CLP诱导的脓毒症炎症小鼠模型[18],其中细胞实验采用10 μmol/L Adox,为估算体内等效剂量,按小鼠体质量与体液分布容积换算,预期小鼠血浆浓度约5~12 μmol/L,以小鼠体质量为20 g,循环体积为12 mL为例,体内剂量应为:10 μmol/L×12 mL/20g=1.59 mg/kg Adox,本文体内实验选用浓度为2 mg/kg,二者处于同一数量级,体内外给药剂量具有较好一致性。在此基础上评价了Adox在脓毒症炎症模型中的抗炎作用,并系统分析了其对脓毒症炎症反应及组织损伤的影响。

3.1 脓毒症炎症反应伴随甲基化代谢重编程和H3K4me3修饰上调

本研究发现,100 ng/mL LPS刺激后显著上调了RAW264.7和THP-1细胞SAHH蛋白表达,同时增加了SAM水平及SAM/SAH比值,升高H3K4me3修饰水平,激活NF-κB信号通路,并促进TNF-α和IL-6的表达与释放。在CLP诱导的脓毒症小鼠肺、脾组织中,H3K4me3表达升高,NF-κB信号通路被激活。提示炎症状态下SAHH表达上调,伴随SAM/SAH比值升高,为H3K4me3修饰提供有利的甲基化代谢环境。这一发现与既往研究一致。相关研究指出,H3K4me3可促进NF-κB、TNF-α等炎症基因的转录活化[21-22]。与此同时,激活后的NF-κB又能够募集组蛋白甲基转移酶至靶基因启动子区域,促进H3K4me3沉积,从而进一步增强炎症基因表达[23]。提示H3K4me3与NF-κB之间可能存在协同放大效应,共同参与脓毒症炎症反应的调控。

3.2 Adox通过调控SAHH、SAM/SAH代谢及H3K4me3修饰发挥抗炎作用

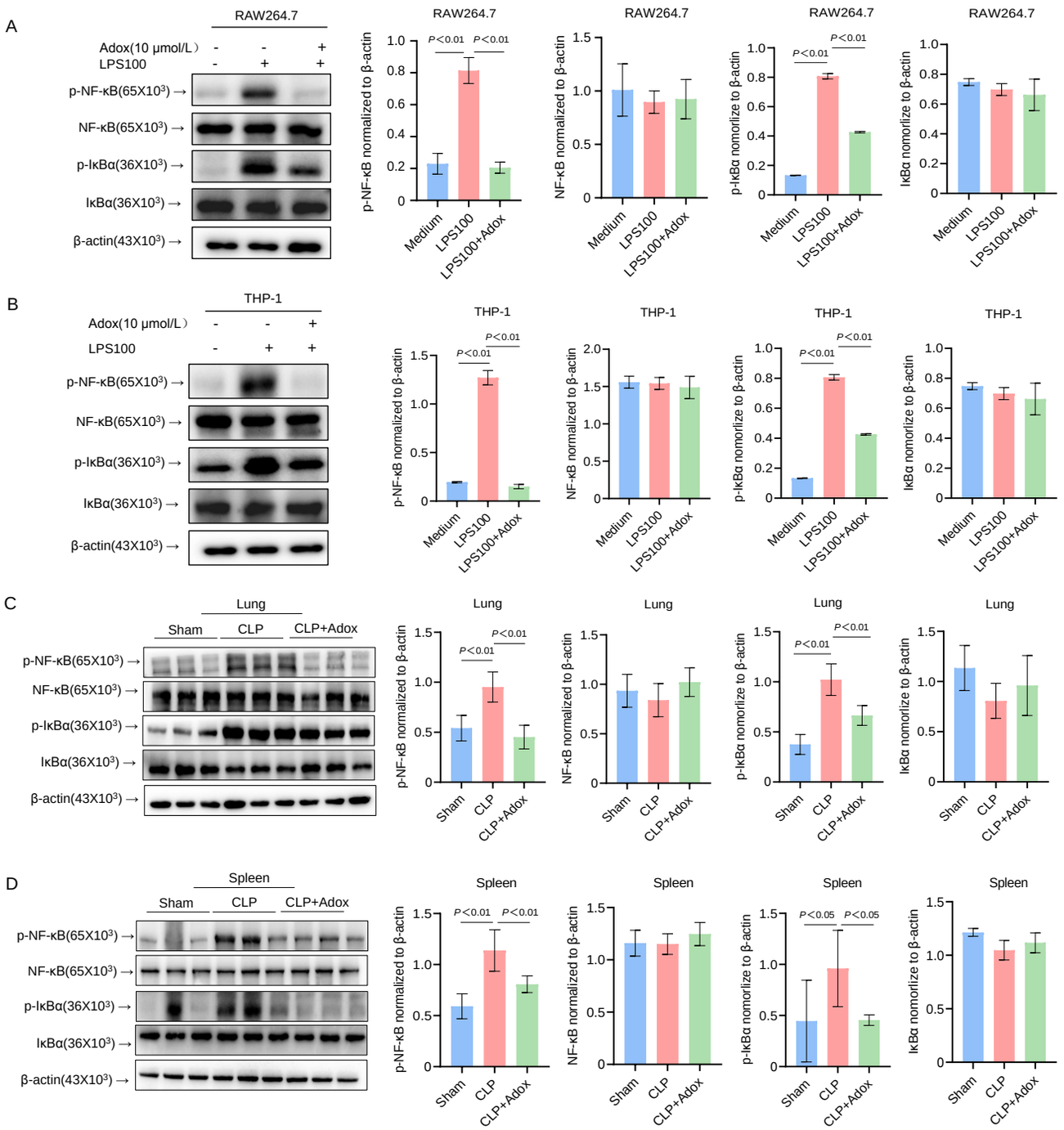
Adox是经典的SAHH抑制剂,可阻断SAHH水解,导致细胞内SAH蓄积[24]。SAH作为甲基转移酶的



A and B: Protein expression levels of SAHH in RAW264.7 (A) and THP-1 (B) cells of the Medium group, the LPS stimulation group, and the LPS100+Adox group; C to E: Contents of SAM (C) and SAH (D) and SAM/SAH ratio (E) in RAW264.7 cells; F and G: Protein H3K4me3 level in RAW264.7 (F) and THP-1 (G) cells; H and I: H3K4me3 expression in the lung (H) and spleen (I) tissues of CLP mice

图5 Western blot检测SAHH、H3K4me3蛋白表达情况、ELISA检测SAM、SAH代谢变化（n=4，$\bar{x}\pm s$）

Figure 5 Protein expression of SAHH and H3K4me3 by Western blot and metabolic changes in SAM and SAH by ELISA（n=4，$\bar{x}\pm s$）



A and B: Expression of NF-κB signaling pathway-related proteins in RAW264. 7 (A) and THP-1 (B) of the Medium group, the LPS stimulation group, and the LPS100+Adox group; C and D: Expression of NF-κB signaling pathway-related proteins in the lung (C) and spleen (D) tissues of CLP mice

图6 Western blot检测NF-κB信号通路相关蛋白表达情况(n=3,$\bar{x}\pm s$)

Figure 6 Detection of the expression levels of NF-κB signaling pathway-related proteins by Western blot (n=3,$\bar{x}\pm s$)

产物抑制剂,可反馈性抑制甲基转移酶活性,降低SAM/SAH比值,从而减少H3K4me3等甲基化修饰的沉积[25]。本研究发现,Adox干预后SAHH和SAM水平降低,SAM/SAH比值降低并伴随H3K4me3修饰下降,提示其能够重塑甲基化代谢稳态并降低细胞甲基化潜能,进而抑制H3K4me3等转录激活性修饰。与此同时,NF-κB和IκBα磷酸化水平受到抑制,炎症因子释放减少,肺和脾组织损伤明显改善。

3.3 Adox与同类SAHH抑制剂及抗炎药物的对比分析

相较于同类SAHH抑制剂,Adox是蛋白甲基化研究中应用较广泛的工具化合物[26]。另一种SAHH抑制剂3-deazaadenosine(3-DA)同样具有抗炎作用,但其机制更侧重于直接抑制MEK1/2和IKKα/β激酶活性,阻断AP-1和NF-κB信号通路[17]。相比之下,本研究提示Adox可能通过调控SAHH介导的SAM/SAH代谢及H3K4me3修饰影响炎症相关基因转录,为脓毒症抗炎干预提供了新的研究思路。与糖皮质激素及直接NF-κB抑制剂等传统抗炎策略相比,Adox并非直接抑制炎症信号,而是通过调控甲基化代谢及表观遗传修饰影响炎症基因转录。但其作用范围较广,目前仍主要作为机制研究工具,其安全性、药代动力学特征及临床应用价值仍需进一步评价。

3.4 研究局限性及未来方向

本研究仍存在一定局限性。①动物实验仅观察了CLP术后6 h这一急性炎症时相,未能动态评价Adox对脓毒症不同病程阶段炎症反应及器官损伤的影响,也未开展生存率评价,因此其长期保护作用仍需进一步验证。②主要检测了H3K4me3修饰水平,尚未系统评估Adox对其他组蛋白甲基化修饰(如H3K27me3、H3K9me3)的影响,其表观遗传调控谱仍有待进一步完善。③主要基于药理学干预和相关性分析,尚未采用siRNA、CRISPR/Cas9等策略进一步验证SAHH在Adox抗炎作用中的关键作用,也未进一步结合ChIP-qPCR等技术检测炎症相关基因启动子区域H3K4me3富集及NF-κB结合变化,以明确SAHH-SAM/SAH代谢改变、H3K4me3修饰与NF-κB信号通路之间的因果关系。④目前Adox作为SAHH抑制剂仍主要应用于基础研究,其在脓毒症中的药代动力学特征、安全性及最佳给药方案尚不明确。未来可结合多时间点、多剂量动物实验及药效学评价,进一步明确其治疗窗,并探索其与现有抗炎治疗策略联合应用的可行性,为临床转化提供实验依据。

综上所述,本研究发现脓毒症体内外模型中,SAHH表达上调、SAM/SAH比值升高,并伴随H3K4me3修饰水平增加及NF-κB信号通路活化,提示甲基化代谢重编程、表观遗传调控与炎症信号转导可能共同参与脓毒症炎症反应的发生发展。Adox干预后能够降低SAM/SAH比值,促进甲基化代谢稳态恢复,抑制H3K4me3修饰水平及NF-κB信号通路活化,从而减轻炎症因子释放和组织损伤。尽管上述结果存在一定局限,但本研究仍为理解脓毒症代谢-表观遗传调控机制提供了新的视角,并为靶向SAHH-SAM/SAH代谢通路干预脓毒症炎症反应提供了实验依据。

利益冲突 本文所有作者声明不存在利益冲突

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(收稿:2026-06-05;修回:2026-07-09)

(编辑 黄 晟)