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在线全文

循环组蛋白诱导内皮功能障碍致脓毒症急性呼吸窘迫综合征的机制研究*

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【摘要】 **目的** 揭示组蛋白引起内皮功能障碍致脓毒症急性呼吸窘迫综合征的机制，为靶向组蛋白治疗脓毒症急性呼吸窘迫综合征提供实验依据。**方法** 体外实验首先采用梯度浓度组蛋白刺激人脐静脉内皮细胞（human umbilical vein endothelial cells, HUVEC），探索体外最佳刺激浓度。进而采用组蛋白刺激HUVEC 24 h进行造模，将细胞分为：①空白对照组、②空白对照+瑞沙托维（Toll样受体4抑制剂）干预组、③组蛋白刺激组、④组蛋白+瑞沙托维干预组。使用流式细胞术测定HUVEC凋亡情况，Western blot法测定内皮细胞VE-cadherin表达情况，共聚焦荧光显微镜拍摄评估内皮细胞间黏着连接的完整性。体内实验采用6～8周22~25 g雄性C57BL/6小鼠，分别通过盲肠结扎穿孔及尾静脉注射50 mg/kg组蛋白构造脓毒症模型。实验动物分为：①空白对照、②空白对照+瑞沙托维干预组、③盲肠结扎穿孔模型组、④盲肠结扎穿孔+瑞沙托维干预组、⑤组蛋白模型组、⑥组蛋白+瑞沙托维干预组。24 h后，采用ELISA法检测各组小鼠血清中白细胞介素-6（interleukin-6, IL-6）及肿瘤坏死因子-α（tumor necrosis factor-α, TNF-α）质量浓度；使用Western blot法检测肺脏组织VE-cadherin表达情况；HE染色观察各组小鼠肺脏组织病理变化。在小鼠处死前30 min，于尾静脉注射伊文思蓝。处死小鼠后，取肺脏组织评估各组小鼠每单位质量肺脏中伊文思蓝染料浓度，计算肺脏内皮漏出率，评估肺脏内皮屏障完整性。**结果** 体外实验结果提示，与对照组相比，组蛋白刺激下HUVEC凋亡增加（$P<0.05$），VE-cadherin表达减少（$P<0.05$），内皮细胞间黏着连接完整性被破坏；瑞沙托维可显著抑制组蛋白诱导的HUVEC凋亡及VE-cadherin表达减少，维持内皮细胞间黏着连接完整性。体内实验中，瑞沙托维可有效缓解盲肠结扎穿孔及组蛋白诱导的脓毒症小鼠血清中IL-6及TNF-α质量浓度增高，减轻小鼠肺脏组织VE-cadherin表达的下调（$P<0.05$），降低小鼠肺脏血管内皮通透性，改善小鼠肺脏组织病理损伤。**结论** 组蛋白通过与Toll样受体4结合导致血管内皮细胞表面VE-cadherin表达下降，破坏细胞间黏着连接完整性，引起肺脏组织病理损伤；使用Toll样受体4抑制剂可阻断组蛋白诱导的脓毒症急性呼吸窘迫综合征。

【关键词】 脓毒症 急性呼吸窘迫综合征 内皮功能障碍 循环组蛋白 Toll样受体4

Mechanism of Extracellular Histone-Induced Endothelial Dysfunction Leading to Sepsis-Induced Acute Respiratory Distress Syndrome

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【**Abstract**】 **Objective** Sepsis-induced acute respiratory distress syndrome (ARDS) is an independent risk factor for mortality in critically ill septic patients. However, effective therapeutic targets are still unavailable due to the lack of understanding of its unclear pathogenesis. With increasing understanding in the roles of circulating histones and endothelial dysfunction in sepsis, we aimed to investigate the mechanism of histone-induced endothelial dysfunction leading to sepsis-induced ARDS and to provide experimental support for histone-targeted treatment of sepsis-induced ARDS. **Methods** First of all, *in vitro* experiments were conducted. Human umbilical vein endothelial cells (HUVEC) were stimulated with gradient concentrations of histones to explore for the optimal stimulation concentration *in vitro*. Then, HUVEC were exposed to histones at an optimal concentration with or without resatorvid (TAK-242), a selective inhibitor of Toll-like receptor 4 (TLR4), for 24 hours for modeling. The cells were divided into 4 groups: 1) the blank control group, 2) the blank control+TAK-242 intervention group, 3) the histone stimulation group, and 4) the histone+TAK-242 intervention group. HUVEC apoptosis was determined by flow cytometry, VE-Cadherin expression in endothelial cells was determined by Western blot, and the integrity of adhesion connections between endothelial cells was evaluated with confocal fluorescence microscopic images. Male C57BL/6 mice aged 6-8 weeks and weighing 22-25 g were used for the *in vivo* experiment. Then, the mice were given cecal ligation and puncture (CLP) as well as histone injection at 50 mg/kg via the tail vein for sepsis modeling. The experimental animals were divided into 6 groups: 1) the blank control group, 2) the blank control+TAK-242 intervention group, 3) the CLP model group, 4) the CLP+TAK-242 intervention group, 5) the histone model group, and 6) the histone+TAK-242 intervention group. After 24 h, the concentrations of serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were determined using ELISA kits. Western blot was performed to determine the expression of vascular endothelial (VE)-cadherin in the lung tissue. Hematoxylin and eosin (HE) staining was performed to observe the pathological changes in the lung tissue of the mice.

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Evans Blue was injected via the tail vein 30 min before the mice were sacrificed. Lung tissue was collected after the mice were sacrificed. Then, the concentrations of Evans blue dye per unit mass in the lung tissue from mice of different groups were evaluated, the rates of pulmonary endothelial leakage were calculated, and the integrity of the pulmonary endothelial barrier was evaluated. **Results** The results of the *in vitro* experiment showed that, compared with those of the control group, HUVEC apoptosis was significantly increased under histone stimulation (P<0.05), the expression of VE-cadherin was decreased (P<0.05), and the integrity of adherens junctions between endothelial cells was damaged. TAK-242 can significantly inhibit histone-induced HUVEC apoptosis and VE-cadherin expression reduction and maintain the integrity of adherens junctions between endothelial cells. According to the findings from the *in vivo* experiments, in mice with CLP-induced and histone-induced sepsis, TAK-242 effectively alleviated the increase in serum concentrations of IL-6 and TNF-α, reduced the downregulation of VE-cadherin expression in the lung tissue (P<0.05), decreased endothelial permeability of the lung vessels, and improved pathological injury in the lung tissue. **Conclusion** By binding to TLR-4, histone decreases VE-cadherin expression on the surface of vascular endothelial cells, disrupts the integrity of intercellular adherens junctions, and triggers pathological damage to lung tissue. Using TLR-4 inhibitors can prevent sepsis-induced ARDS in histone-induced sepsis.

【Key words】 Sepsis　Acute respiratory distress syndrome　Endothelial dysfunction　Extracellular histone　Toll-like receptor 4

急性呼吸窘迫综合征(acute respiratory distress syndrome, ARDS)是肺内和肺外因素在短时间内引起的以弥漫性肺泡损伤为特征的急性低氧性呼吸衰竭综合征,病理表现主要包括肺水肿、透明膜形成、肺泡出血和炎症[1-2]。脓毒症是ARDS最主要的肺外原因,约占ARDS病例的32%[3]。研究表明,脓毒症导致的ARDS较其他因素导致的ARDS更为严重,预后更差,病死率更高,ICU中约有54.8%的脓毒症患者发生ARDS合并症,而合并了ARDS的脓毒症患者死亡率高达71%[3]。脓毒症疾病本身的复杂性以及ARDS病情发展迅速及病变的严重性,使得常用的治疗手段对脓毒症相关ARDS疗效甚微[4]。

循环组蛋白是一种新近发现的带正电荷的损伤相关分子模式。在脓毒症、严重创伤、急性胰腺炎等危重症发生时,组蛋白自核内释放入血,致使患者血清中循环组蛋白浓度显著升高。临床数据表明,创伤性肺损伤患者的循环组蛋白在4 h内立即从10 μg/mL升高到230 μg/mL,并在24 h左右达到峰值[5]。循环组蛋白浓度与脓毒症[6]及ARDS[7]患者器官衰竭严重程度及死亡率呈正相关。然而,循环组蛋白在脓毒症相关ARDS中的作用机制尚不明确。本研究旨在初步探究组蛋白诱导的内皮细胞黏着连接破坏在脓毒症相关ARDS发生及进展中的作用,为靶向循环组蛋白治疗脓毒症相关ARDS提供理论依据。

1　材料与方法

1.1　主要抗体与试剂

组蛋白(Sigma公司, H5505, 小牛胸腺来源), Toll样受体4(Toll-like receptor 4, TLR-4)抑制剂瑞沙托维(TAK-242)(Sigma公司, 614316), 伊文思蓝(Sigma公司, E2129), VE-cadherin抗体(Cell Signaling Technology公司, 2500), CDH5抗体(Boster Bio公司, A02632-2), β-tubulin抗体(Cell Signaling Technology公司, 2146)

1.2　细胞培养

37 ℃、体积分数5%CO_2恒温培养箱用于原代人脐静脉内皮细胞(human umbilical vein endothelial cells, HUVEC)(ATCC公司购入)培养,增殖至80%～90%时进行细胞传代,所有细胞实验均使用第4～6代细胞进行。

1.3　细胞实验分组及干预

取增殖至80%左右的原代HUVEC细胞,首先分为6组,使用0、25 μg/mL、50 μg/mL、75 μg/mL、100 μg/mL及200 μg/mL梯度浓度组蛋白进行刺激,通过观察HUVEC细胞间黏着连接分子VE-cadherin表达量变化(Western blot)、细胞间黏着连接完整性(免疫荧光染色)以及细胞凋亡情况(流式细胞术),确定体外实验最佳组蛋白刺激浓度。检测方法见1.4～1.6小节。

取增殖至80%左右的原代HUVEC细胞,分为4组:①空白对照组(Control group),仅用生理盐水处理细胞36 h;②空白对照+瑞沙托维干预组(Control+TAK-242 group),仅用瑞沙托维(10 μmol/L)处理细胞36 h;③组蛋白造模组(Histone group),先用生理盐水预处理12 h,再加入50 μg/mL组蛋白,持续刺激细胞24 h;④组蛋白造模+瑞沙托维干预组(Histone+TAK-242 group),先用瑞沙托维(10 μmol/L)预处理12 h,其余同③组。然后进行1.4～1.6小节的检测。

1.4　Western blot检测VE-cadherin表达

将各组细胞孔板置于冰上,吸去培养基,使用预冷的PBS充分洗涤后,向各孔加入适量含PMSF的裂解液,并转移至预冷样本管中。将上述裂解样本于冰上静置30 min。在4 ℃低温下,14 500 r/min离心15 min,转移各管上清至新离心管中。使用BCA试剂盒检测各样本提取蛋白浓度。按体积比,向各组蛋白液中加入上样缓冲液,置于

95 ℃加热10 min。煮样结束后,立即将蛋白溶液置于冰上冷却备用。目标蛋白抗体为VE-cadherin(1∶1 000),内参蛋白采用β-tubulin(1∶10 000)。利用ImageJ软件分析目标蛋白灰度值与内参蛋白灰度值的比值,计算VE-cadherin相对表达量。

1.5 免疫荧光染色检测HUVEC细胞间黏着连接完整性

弃各组细胞培养基,使用预冷PBS充分洗涤。加入体积分数4%多聚甲醛固定细胞15 min后,再次使用预冷的PBS洗涤3次。样品经通透化、封闭处理、再次洗涤后,加入含1%BSA的PBST中稀释的VE-cadherin(1∶200),置于4 ℃孵育过夜。孵育后,将样品用PBST剧烈洗涤两次,再用PBS洗涤3次。向样品中加入含1%BSA〔添加1 μg/mL DAPI(1∶100)〕的PBST中稀释的二抗(1∶500),室温孵育60 min。孵育完毕,再次使用PBST充分洗涤样本后,置于共聚焦显微镜下观察各样本细胞间黏着连接完整性。

1.6 流式细胞术检测HUVEC细胞凋亡情况

收集各组HUVEC培养孔细胞及培养基至2 mL离心管中,使用细胞凋亡试剂盒,对各组细胞凋亡情况进行检测。

1.7 动物模型及分组干预

6~8周龄雄性SPF级C57BL/6小鼠,体质量22~25 g,均购于成都集萃药康生物科技股份有限公司。实验前,将所有小鼠于温度18~25 ℃、湿度适宜、空气流通、日夜节律12 h,小鼠自由摄食、饮水的环境中分笼饲养1周。本研究的动物实验符合国家实验动物福利伦理标准,并经四川大学华西医院动物伦理委员会批准,批准号:20220714002。

将30只小鼠随机分为6组(每组5只),分别予以以下处理:①空白对照组 (Control group):尾静脉注射100 μL生理盐水,注射1 h后再予以尾静脉注射生理盐水100 μL;②空白对照+瑞沙托维干预组 (Control+TAK-242 group):尾静脉注射100 μL生理盐水,注射1 h后尾静脉予以瑞沙托维100 μL(3 mg/kg);③盲肠结扎穿孔模型组 (CLP group):于小鼠腹正中线行一长约1 cm纵向切口进入腹腔,自盲肠末端到回、盲肠衔接处计为盲肠总长且总长的75%处为缝线结扎部位,使用21 G针头穿刺结扎段肠壁,使结扎段盲肠内容物溢出后,回纳腹腔内容物,关腹、补液;④盲肠结扎穿孔+瑞沙托维干预组 (CLP+TAK-242 group):使用CLP构造脓毒症模型1 h后,尾静脉予以瑞沙托维100 μL(3 mg/kg);⑤组蛋白模型组 (Histone group):尾静脉注射100 μL组蛋白溶液(50 mg/kg),注射1 h后尾静脉予以生理盐水100 μL;⑥组蛋白+瑞沙托维干预组 (Histone+TAK-242 group):尾静脉注射100 μL组蛋白溶液(50 mg/kg),注射1 h后尾静脉予以瑞沙托维100 μL(3 mg/kg)。24 h后记录各组小鼠存活情况,处死小鼠并收集血清检测小鼠炎症指标,取肺脏样本进行组织学检查、肺脏内皮通透性测定,具体方法见1.8~1.10小节。另取肺脏样本进行液氮速冻,加入适量含PMSF的裂解液研磨裂解,取裂解样本,Western blot检测小鼠肺脏组织中VE-cadherin表达,具体方法同1.4小节。

1.8 小鼠炎症指标测定

取小鼠血液样本,室温静置1 h,4 ℃条件下3 000 ×*g*离心15 min,转移上清至样品管中,根据相应ELISA试剂盒流程,检测各组小鼠血清中炎症因子IL-6及TNF-α水平。

1.9 小鼠肺脏HE染色

肺脏组织经固定液固定24 h后进行冲洗、脱水、石蜡包埋。将修整好的蜡块使用石蜡切片机切至厚度4 μm后烤片、制片。制片完成后予以脱蜡至水、预处理,再予以苏木素及伊红染色后脱水封片,镜下观察,对肺脏组织结构采取Smith评分方法,对肺脏组织的肺水肿、肺泡及间质炎症、肺泡及间质出血、肺不张及透明膜形成,分别予以0~4分半定量分析,总肺损伤评分为上述各项之和。每只小鼠取10个高倍视野,计算平均值。

1.10 小鼠肺脏内皮通透性测定

根据RADU等[8]所报道的方法,在小鼠处死前30 min,经尾静脉注射0.5%的伊文思蓝溶液200 μL。使用颈椎脱位法处死小鼠后,迅速收集小鼠肺脏至1.5 mL试管中,并称取组织质量。向各样本中加入500 μL甲酰胺溶液,置于55 ℃中水浴孵育48 h,于610 nm处测取各样本甲酰胺溶液吸光度,计算以小鼠每毫克肺脏组织所含的伊文思蓝染料含量,评估小鼠内皮屏障渗透性。

1.11 统计学方法

使用GraphPad Priam 9软件进行统计学分析。定量数据均使用$\bar{x} \pm s$进行描述,各实验组间的多组比较采用单因素方差分析(ANOVA)进行,进一步组间两两比较使用SNK法、Tukey's检验、Dunnett's检验等方法,$P<0.05$为差异有统计学意义。

2 结果

2.1 组蛋白通过浓度梯度依赖性诱导HUVEC细胞凋亡及细胞间黏着连接破坏

在体外实验中,组蛋白可通过浓度梯度依赖的特性诱导HUVEC细胞间黏着连接分子VE-cadherin的表达下降(图1)。进一步选择其中的两个质量浓度(50 μg/mL和100 μg/mL组蛋白溶液),对VE-cadherin进行免疫荧光共聚焦染色发现,组蛋白刺激引起HUVEC细胞间黏着连接的完整性的破坏,也是质量浓度高者更明显(图2)。此外,组蛋白也以浓度梯度特性诱导内皮细胞发生凋亡

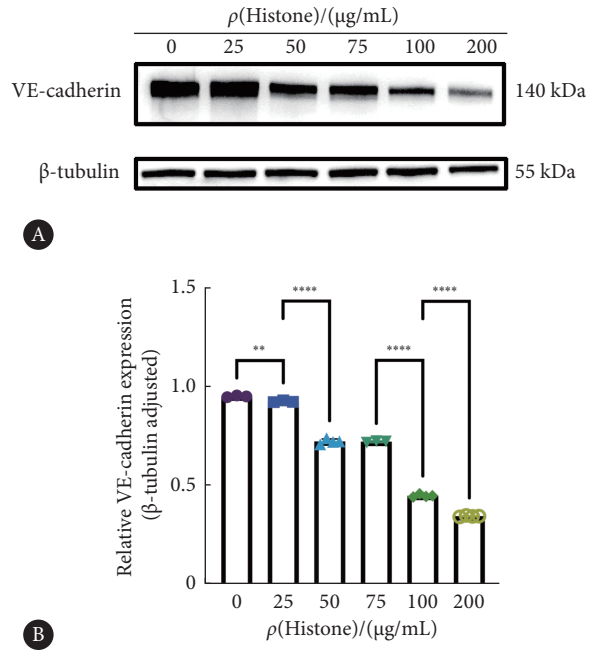

图 1　组蛋白诱导人脐静脉内皮细胞表达VE-cadherin下降

Fig 1　Histone induced decreased expression of VE-cadherin in HUVEC in a dose-dependent manner

** $P<0.01$, **** $P<0.000\,1$. $n=4$.

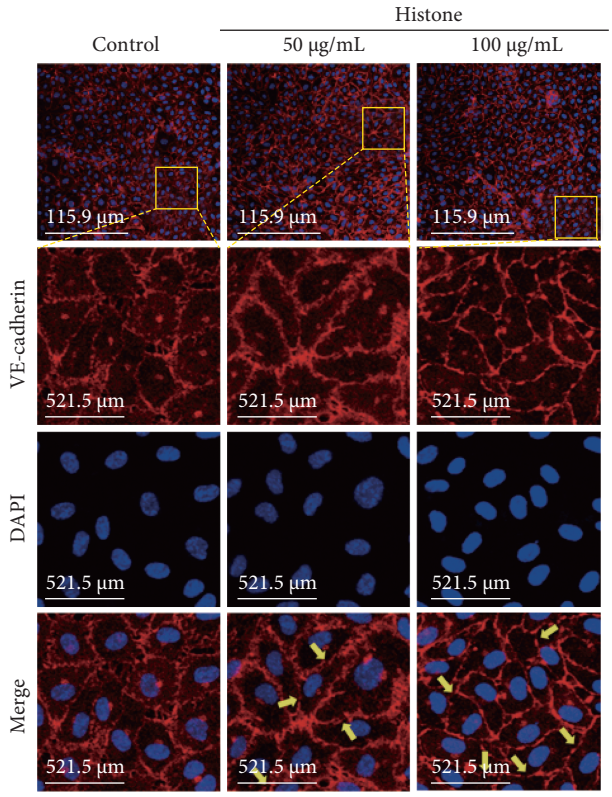

图 2　组蛋白破坏人脐静脉内皮细胞间黏着连接完整性

Fig 2　Histone disrupted the integrity of VE-cadherin of HUVEC in a dose-dependent manner

The yellow arrows indicate the point of broken integrity of the adhesion connection between cells (continuity break point).

(图3)。由此可见, 使用50 μg/mL的组蛋白溶液刺激原代HUVEC细胞可明显破坏HUVEC细胞间黏着连接完整性, 减少HUVEC细胞VE-cadherin表达, 并一定程度诱导内皮细胞凋亡; 故而我们选择50 μg/mL组蛋白溶液进行后继体外实验。

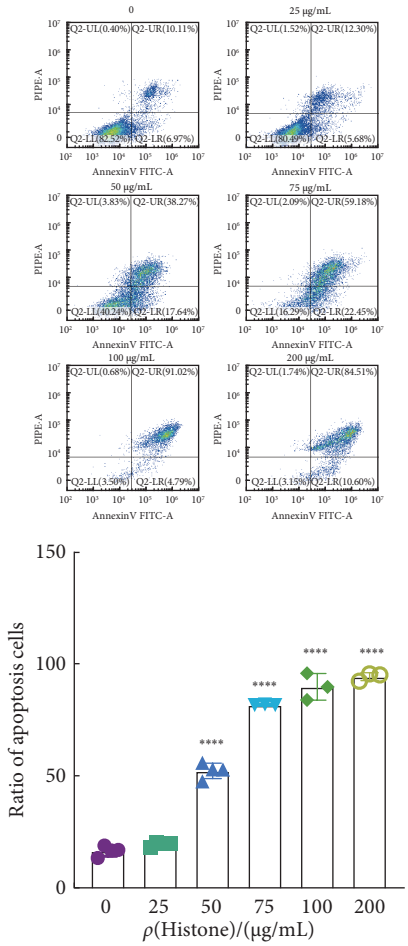

图 3　组蛋白诱导人脐静脉内皮细胞凋亡

Fig 3　Histone induced HUVEC apoptosis in a dose dependent manner

**** $P<0.000\,1$, vs. 0 μg/mL histone. $n=4$.

2.2　TLR-4受体抑制剂瑞沙托维有效拮抗组蛋白诱导的HUVEC细胞凋亡及黏着连接破坏

预先使用TLR-4抑制剂瑞沙托维孵育12 h, 可有效缓解组蛋白诱导的VE-cadherin表达下调(图4), 维持HUVEC细胞间黏着连接完整性(图5), 并减少组蛋白诱导的HUVEC细胞凋亡(图6)。此外, 对比Control组及Control+TAK-242组结果可见, 单独使用TLR-4抑制剂TAK-242可一定程度上调VE-cadherin表达(图4), 增强内皮屏障稳定性(图5), 并减少HUVEC细胞凋亡(图6)。

2.3　TLR-4受体抑制剂瑞沙托维对脓毒症相关ARDS的影响

如图7所示, 盲肠结扎穿孔(CLP模型组)及尾静脉注射组蛋白(组蛋白模型组)均显著升高脓毒症小鼠血清中

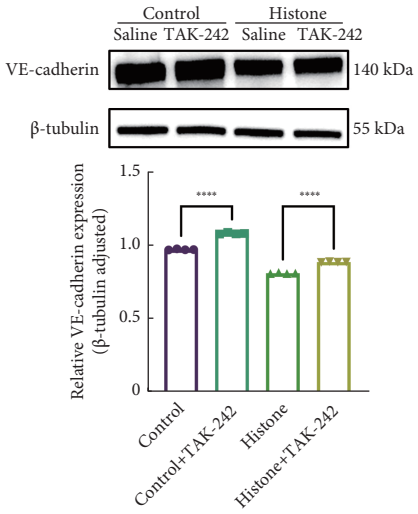

图 4 TLR4抑制剂TAK-242缓解组蛋白诱导人脐静脉内皮细胞VE-cadherin低表达

Fig 4 TAK-242, a TLR-4 inhibitor, significantly alleviated histone-induced low expression of VE-cadherin in HUVEC

**** $P<0.000\,1$. $n=4$.

IL-6及TNF-α质量浓度。相较于这两种脓毒症模型组，尾静脉使用TAK-242均显著降低小鼠血清中IL-6及TNF-α质量浓度。在两种脓毒症模型中，小鼠肺脏组织内皮通透性均显著增加；而TAK-242有效拮抗了这种内皮高通透性。随后，Western blot实验发现TAK-242显著缓解CLP和组蛋白诱导的脓毒症小鼠肺脏组织VE-cadherin低表达。此外，与Control组相比，Control+TAK-242小鼠组肺脏组织的VE-cadherin表达有所增加(图8)。

如图9所示，与空白对照组相比，盲肠结扎穿孔模型组及组蛋白模型组小鼠肺脏间质均出现明显的炎症细胞浸润、肺泡间隔增厚、肺间质出血，可见肺泡充血、水肿、肺不张等；使用TAK-242可显著缓解上述病理损伤(图9A、图9C)。使用Smith评分对肺脏损伤进行定量评估可见：脓毒症小鼠使用TAK-242后均显著降低了肺脏损伤评分($9.181\,8\pm0.873\,9$ vs. $2.818\,2\pm0.750\,8$, $P<0.000\,1$; $7.818\,2\pm0.981\,7$ vs. $3.181\,8\pm0.981\,7$, $P<0.000\,1$)。

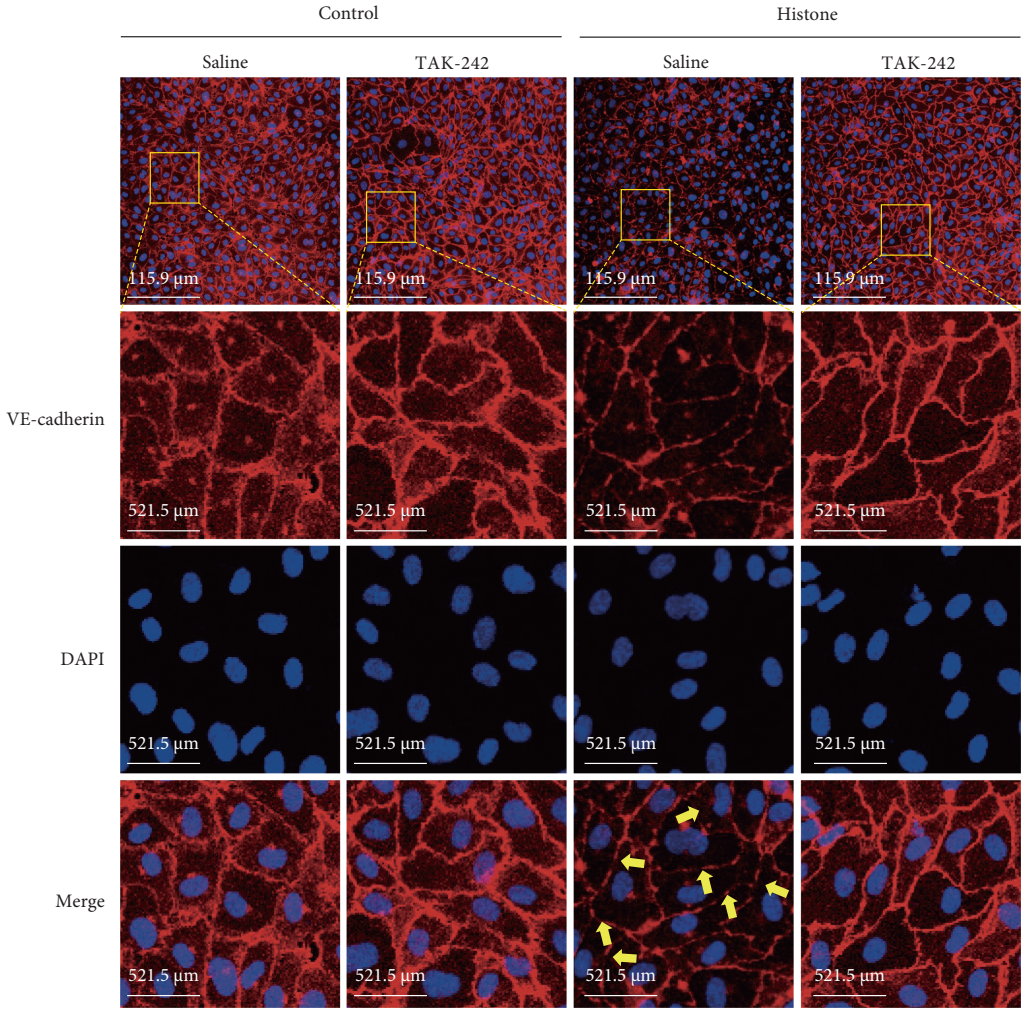

图 5 TAK-242拮抗组蛋白破坏人脐静脉内皮细胞VE-cadherin完整性

Fig 5 TAK-242 significantly alleviated histone-induced damage to VE-cadherin integrity in HUVEC

The yellow arrows indicate the point of broken integrity of the adhesion connection between cells (continuity break point).

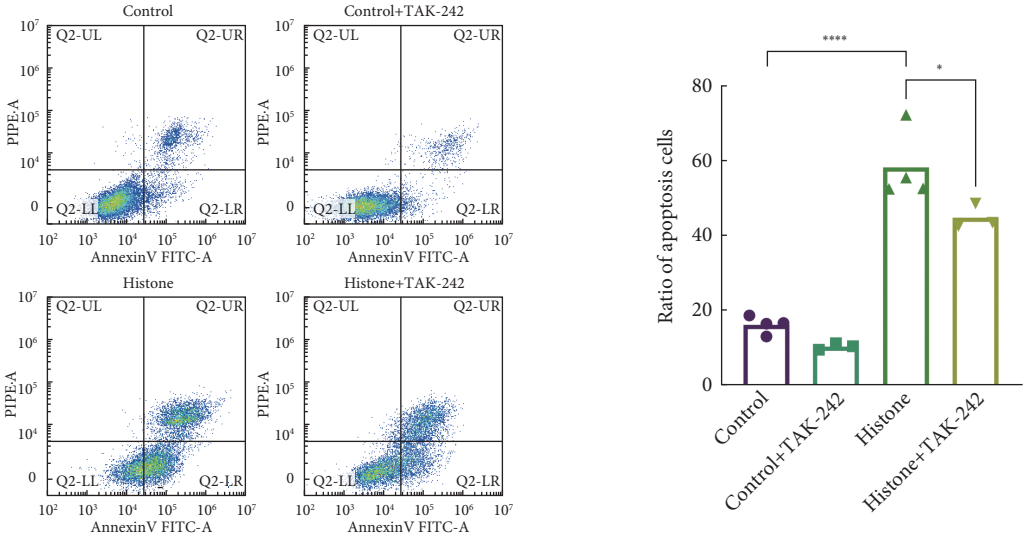

图 6 TAK-242显著改善组蛋白诱导人脐静脉内皮细胞凋亡

Fig 6 TAK-242 significantly remitted histone-induced HUVEC apoptosis

* P<0.05, **** P<0.000 1. n=4.

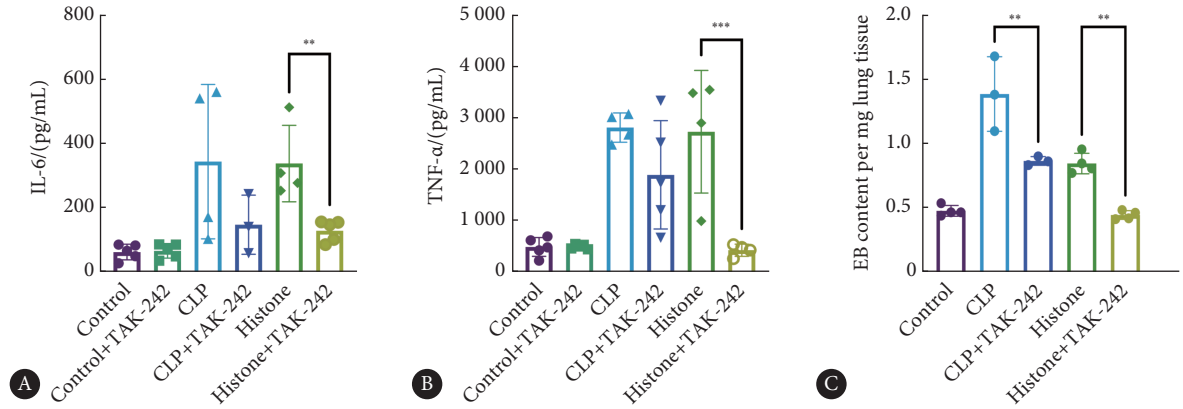

图 7 TAK-242减轻脓毒症小鼠血清炎症水平并维持了肺脏内皮屏障完整性

Fig 7 TAK-242 antagonized inflammatory states and hyperpermeability in septic murine models induced by cecum ligation and puncture and and histone

A and B, IL-6 and TNF-α levels in serum were determined using ELISA kits. C, Relative levels of Evans Blue Dye (EBD) absorbance at 610 nm in the lungs of sham and CLP mice with or without TAK-242 administration. ** P<0.01, *** P<0.001. n=4.

3　讨论

ARDS作为脓毒症患者最常见的并发症之一，合并ARDS的脓毒症患者常具有较高的死亡率及较低的呼吸机脱机成功率[3]。目前，脓毒症相关ARDS的发病机制仍不完全明确。现有的治疗方案难以在较短的窗口期，通过予以肺泡活性剂、激素、抗生素、中性粒细胞弹性酶抑制剂等常规药物，或是通过予以机械辅助通气等器械治疗，有效控制脓毒症相关ARDS患者体内的全身性炎症反应[3]。

近年来，关于ARDS的相关生物标志物得到巨大发展。例如，糖基化终产物受体可作为Ⅰ型肺泡上皮损伤标志[9]；IL-1受体拮抗剂（IL-1 receptor antagonist, IL-1RA），作为一种具有抗炎作用的天然物质，其浓度与ARDS患者预后密切相关。研究表明，根据受试者差异增加血浆IL-1RA水平，可有效改善感染性休克患者生存率，但其疗效仍存在显著争议[10-11]。Ang-2作为内皮损伤的指标，同样可作为预测ARDS患者预后及死亡的指标[12]。近年来，研究发现循环组蛋白在脓毒症患者多器官功能障碍的发生及进展中发挥重要作用，是脓毒症潜在的治疗靶点。在生理状态下，组蛋白作为一种构成染色体基本结构核小体的成分蛋白，主要起到稳定染色体结构、调节细胞的转录及翻译工作的作用。在脓毒症[6]、急性胰腺炎[13]、急性肝损伤[14]等多种危重症发生时，一方面，组织细胞可发生凋亡、坏死，导致大量细胞内组蛋白释放进入循环；另一方面，脓毒症患者体内中性粒细胞、巨噬细胞

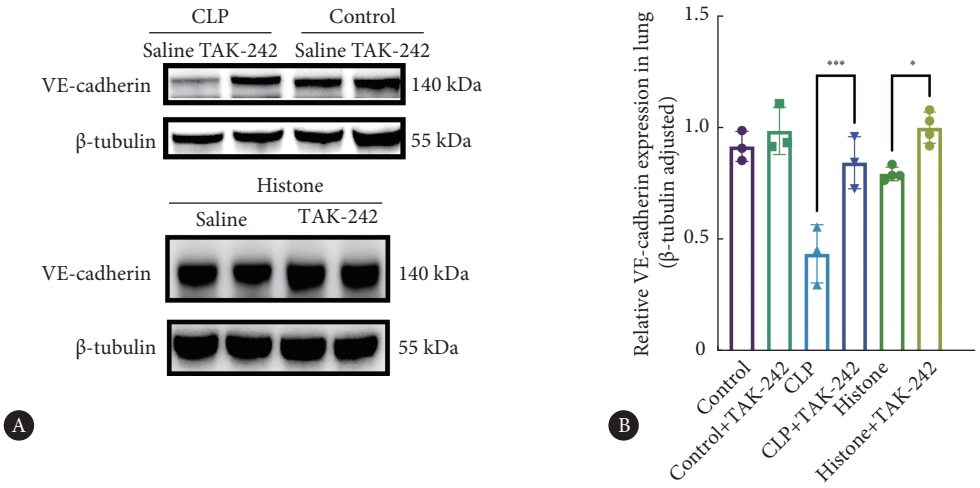

图 8 TAK-242改善脓毒症小鼠肺脏组织中VE-cadherin低表达

Fig 8 TAK-242 improved VE-cadherin expression in the lung tissue of murine septic models induced by cecum ligation and puncture and histone infusion

* $P<0.05$, *** $P<0.001$. $n=4$.

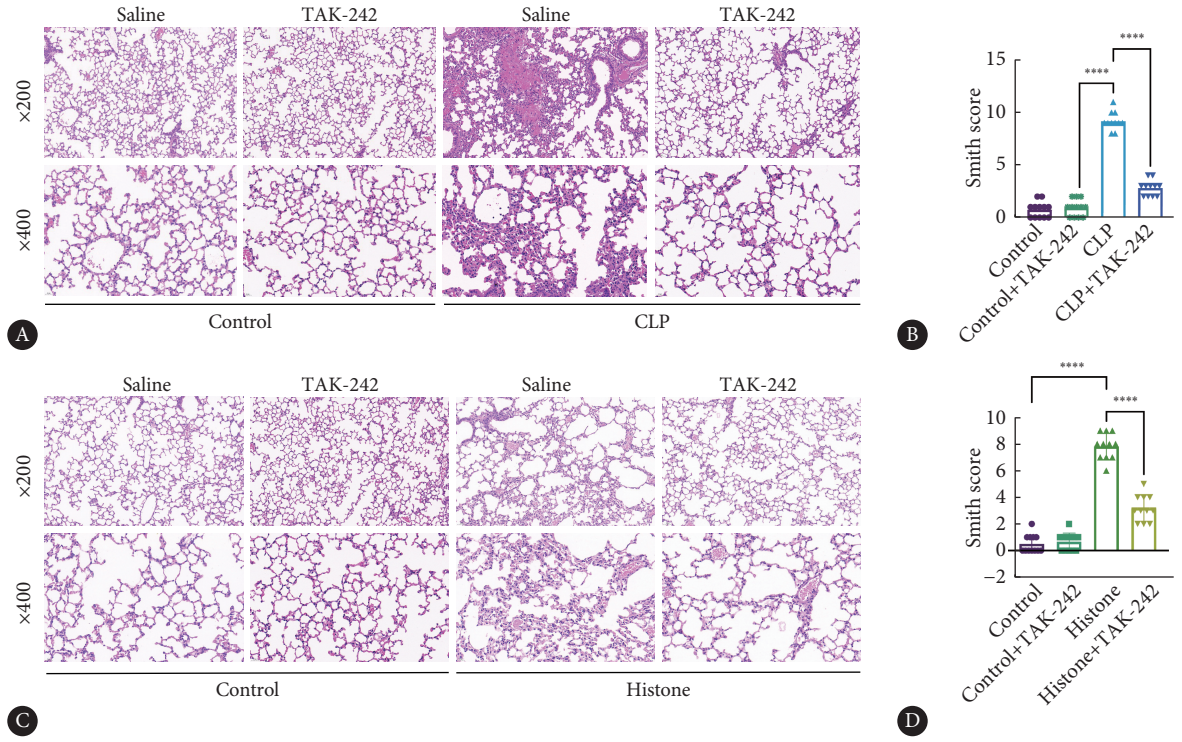

图 9 TLR-4抑制剂TAK-242对脓毒症急性肺损伤小鼠肺脏病理的影响（HE染色）

Fig 9 The effect of TAK-242 on lung pathology in mice with sepsis (HE stains)

A, Representative images of HE staining of lung tissues from sham and CLP mice with or without TAK-242 administration. B, Representative lung injuries were presented by Smith score. C, Representative images of HE staining for sham and histone-infused mice with or without TAK-242 administration. D, Representative lung injuries were presented by Smith score. **** $P<0.000\ 1$. $n=5$.

等固有免疫细胞被激活后，染色体解螺旋形成细胞外诱捕网，促使固有免疫细胞大量释放核内组蛋白入血[15]，使得循环中组蛋白浓度显著上升。临床研究表明，循环组蛋白浓度与脓毒症患者疾病严重程度以及死亡率显著相关[6, 16]。

肺脏毛细血管内皮及肺泡上皮受损是ARDS核心的病理变化。内皮功能障碍的特征是内皮细胞结构和功能的变化，具体包括：渗透性增加导致血管渗漏及水肿形成；细胞表面黏附分子、受体以及相关信号转导分子表达增加；内皮细胞促凝及抗纤溶表型增加等[4]。越来越多的

研究表明,组蛋白与内皮功能障碍有着密切联系。一方面,组蛋白可通过其正电荷与细胞表面磷脂分子结合产生直接细胞毒性,同时引起钙离子内流损伤内皮细胞[17]。另一方面,循环组蛋白可通过活化caspase-1,诱导内皮细胞自噬和凋亡引发内皮屏障破坏[18]。组蛋白还能通过肝素酶途径诱导内皮细胞表面糖萼的降解,增加内皮通透性[17, 19]。此外,组蛋白通过降低内皮细胞间紧密连接及黏着连接相关分子表达,诱导内皮细胞间黏附分子发生磷酸化等修饰,破坏内皮屏障的完整性[17, 20]。值得一提的是,循环组蛋白与内皮细胞表面的Toll受体结合后,可激活下游NF-κB及NLRP3炎症小体等炎症信号通路,诱发炎症风暴及机体凝血功能障碍[21-22]。此前,KIM等的研究结果表明,在体外实验中使用TLR-4抑制剂CLI-09可显著改善组蛋白及内毒素刺激下的人肺动脉内皮细胞黏附分子VCAM-1及ICAM-1高表达,减少炎症因子*TNF-α*、*IL-6*、*IL-8*及*IL-1β* mRNA的表达[23-24],改善了ARDS过程中内皮功能障碍。

本研究首次证实循环组蛋白可通过TLR-4下调内皮细胞VE-cadherin表达,破坏肺脏内皮细胞间黏着的完整性,导致肺血管内皮功能障碍,致使炎性细胞、液体及大分子物质等进入间质间隙和肺间隙,引起肺水肿和换气功能障碍,促进脓毒症相关ARDS发生及进展。抑制TLR-4可通过上调内皮细胞VE-cadherin表达、稳定内皮细胞间黏着连接完整性,维持内皮屏障功能,有效改善脓毒症小鼠肺脏组织病理损伤,降低炎症因子水平。此外,本研究还发现单独予以TLR-4抑制剂TAK-242时,内皮细胞会显著上调黏附分子VE-cadherin的表达。后续,课题组将继续研究组蛋白诱导内皮功能障碍导致脓毒症相关ARDS的具体分子机制,为靶向循环组蛋白治疗脓毒症相关ARDS提供新的实验依据。

*　　*　　*

作者贡献声明　杨汀航负责论文构思、数据审编、研究方法和初稿写作,李育需负责正式分析、经费获取、研究方法、提供资源和初稿写作,苏白海负责论文构思、经费获取、监督指导、初稿写作和审读与编辑写作。所有作者已经同意将文章提交给本刊,且对将要发表的版本进行最终定稿并同意对工作的所有方面负责。

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