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## 奥曲肽通过调节重症急性胰腺炎大鼠肠道免疫功能 发挥肠道保护的功能

张茂, 郑斌, 张显菡

(四川省第四人民医院普外科, 成都 610016)

**[摘要]** 目的: 探讨奥曲肽对重症急性胰腺炎(severe acute pancreatitis, SAP)大鼠肠道免疫反应的调节功能。方法: SAP大鼠造模后给予奥曲肽治疗, 观察奥曲肽对后肠损伤的保护效果。使用HE染色观察肠组织的损伤状态, 检测大鼠血、肠道中炎症因子的表达水平调节的。通过伊文思蓝染色和测量肠组织干湿重比来评价肠道微血管通透性, 最后对肠组织中NLRP3表达情况进行检测。结果: HE染色显示奥曲肽治疗后可明显改善SAP大鼠肠道损伤, 而奥曲肽治疗组血中TNF- $\alpha$ , IL-1 $\beta$ 及髓过氧化物酶(myeloperoxidase, MPO)的含量显著下降, 显著抑制SAP大鼠小肠组织中TNF- $\alpha$ , IL-1 $\beta$ 及MPO的水平。奥曲肽可减弱SAP大鼠肠道通透性升高并降低SAP大鼠肠内NLRP3的表达。结论: 奥曲肽可调节SAP大鼠肠道免疫功能, 奥曲肽对SAP所致肠黏膜损伤有保护作用。

**[关键词]** 奥曲肽; 重症急性胰腺炎; 大鼠; 肠道; 免疫功能

## Octreotide exerts intestinal protective function by regulating intestinal immune function in rats with severe acute pancreatitis

ZHANG Mao, ZHENG Bin, ZHANG Xianchang

*(Department of General Surgery, Sichuan Provincial Fourth People's Hospital, Chengdu 610016, China)*

**Abstract** **Objective:** To investigate the regulatory effect of octreotide on intestinal immune response in rats with severe acute pancreatitis. **Methods:** The rats with severe acute pancreatitis were treated with octreotide to observe the protective effect of octreotide on intestinal injury. HE staining was used to observe the damage status of intestinal tissue and the expression level of inflammatory factors in blood and intestine of rats were detected. Evans blue staining and wet-dry weight ratio of intestinal tissues were used to evaluate intestinal microvascular permeability. Finally, the expression of NLRP3 in intestinal tissues was detected. **Results:** HE staining showed that octreotide treatment could significantly mitigated intestinal injury in rats with severe acute pancreatitis, while levels of TNF- $\alpha$ , IL-1 $\beta$  and MPO in blood of octreotide treatment group decreased significantly, and levels of TNF- $\alpha$ , IL-1 $\beta$

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通信作者 (Corresponding author): 张茂, Email: zhangmaohuu@163.com

and myeloperoxidase (MPO) in intestine tissue were significantly inhibited. **Conclusion:** Octreotide can regulate intestinal immune function in rats with severe acute pancreatitis. Octreotide has protective effect on intestinal mucosal injury caused by severe acute pancreatitis.

**Keywords** octreotide; severe acute pancreatitis; rats; intestine; immune function

急性胰腺炎(acute pancreatitis, AP)是胰腺的急性炎症,是最常见的急腹症之一<sup>[1]</sup>。尽管大多数AP是自限性的,但10%~20%的患者可能发展为重症急性胰腺炎(severe acute pancreatitis, SAP),由于继发性感染和器官衰竭病死率可达30%<sup>[2]</sup>。SAP的发病机制复杂,其确切机制尚未完全阐明<sup>[3]</sup>。肠道是人体最大的免疫器官,肠黏膜屏障功能的损害可导致SAP患者发生局部和全身性脓毒症并发症<sup>[4]</sup>。细菌移位入血导致的内毒素血症和感染引起的肠黏膜免疫功能障碍是SAP患者死亡的主要原因<sup>[5]</sup>。肠屏障在SAP中起重要作用,当肠屏障功能减弱或受损时,细菌可能通过肠道进入无菌腹腔(腹膜)以引起严重并发症<sup>[6]</sup>。因此,通过调节肠道免疫功能,恢复正常肠黏膜屏障,是预防和治疗SAP的有效策略。奥曲肽是一种作用时间长效的合成类生长抑素(somatostatin, SST)类似物,除抑制胰腺分泌外,还具有调节免疫炎症反应和抑制促炎细胞因子释放的作用<sup>[7]</sup>。奥曲肽的抗炎作用表明它可能是SAP的潜在治疗选择,然而奥曲肽在SAP肠炎症应激治疗中的应用尚无定论,推测其在治疗SAP中可能通过调节肠道免疫应答发挥重要的器官保护作用。因此本研究旨在探索奥曲肽对SAP大鼠肠道免疫功能的影响。

## 1 材料与方法

### 1.1 材料

30只雄性SD大鼠[(200±20)g]购自四川大学实验动物中心[SCXK(川)2018-026],大鼠饲养于四川大学华西第四医院动物房[SYXK(川)2018-011],12 h光照、12 h黑暗活动,自由获取食物和水。本实验获得了四川大学华西第四医院实验动物伦理委员会批准。

### 1.2 试剂及仪器

L-精氨酸和伊文思蓝购自美国Sigma公司;奥曲肽注射液(国药准字H20100114)购自北京双鹭药业股份有限公司;异氟烷和异氟烷麻醉装

置购自深圳市瑞沃德生命科技有限公司;多聚甲醛购自成都金山化学试剂有限公司;TNF- $\alpha$ , IL-1 $\beta$ ,及髓过氧化物酶(myeloperoxidase, MPO)ELISA试剂盒购自深圳欣博盛生物科技有限公司;NLRP3抗体及荧光二抗购自美国CST公司;封片剂购自美国Vector公司。倒置荧光显微镜购自日本NIKON公司;Centrifuge低速离心机购自德国Eppendorf公司;酶标仪购自美国BIORAD公司。

### 1.3 造模及治疗

将大鼠随机分为对照组(Control)、SAP模型组(SAP)及奥曲肽治疗组(SAP+octreotide)3组,每组10只。SAP模型组给予3 g/kg剂量的L-精氨酸腹腔注射诱导胰腺炎,连续注射2次,每次间隔1 h。对照组不进行造模,只给予腹腔注射等量生理盐水。奥曲肽治疗组在造模后给予配置好的奥曲肽注射液(10  $\mu$ g/g)背部皮下注射。18 h后取样观测。

### 1.4 HE 染色

在4%甲醛溶液中浸泡肠组织过夜,将其包埋固定切片后,在乙醇和二甲苯中脱水后,使用苏木精-伊红(HE)按照标准步骤进行染色。

### 1.5 ELISA 试验

收集血清和肠组织匀浆液后,根据检测试剂盒的说明书进行测定TNF- $\alpha$ , IL-1 $\beta$ 及MPO的含量。

### 1.6 大鼠肠毛细血管通透性检测

大鼠肠毛细血管通透性改变分别采用肠组织湿/干重实验和肠组织伊文思蓝染色方法进行检测。肠组织湿/干重实验:取大鼠肠组织,用滤纸吸干表面水分后测得重量为湿重,将组织在60  $^{\circ}$ C烘箱中烘72 h至恒重为干重。肠组织湿重与干重的比值提示组织水肿情况;肠组织伊文思蓝染色:大鼠尾静脉注射1%的伊文思蓝40 mg/kg,3 h后处死,取出肠组织分离染色阳性的组织称量计算。

### 1.7 免疫荧光试验

将肠组织的石蜡切片在65℃下烘烤2 h, 分别以100%、95%和80%乙醇浸泡5 min后, 分别用二甲苯I、二甲苯II和二甲苯III脱蜡10 min, 然后放入70%乙醇和蒸馏水中用于梯度水化。切片水合后用PBS洗涤3次, 每次5 min, 加入0.2 mL 3% H<sub>2</sub>O<sub>2</sub>, 孵育10 min阻断内源性过氧化物酶, 然后用PBS洗涤3次, 每次5 min。将切片放入pH=6的柠檬酸修复液中, 在95℃下煮20 min, 然后自然冷却至室温以修复抗原。切片用PBS洗涤3次, 每次5 min, 加入0.3% TritonX-100破膜10 min, 用PBS漂洗3次, 每次5 min, 然后加入5% FBS溶液封闭30 min, 然后将NLRP3一抗体(1:150)加入切片并置于冰箱中4℃过夜。第2天, 将切片加热1 h, 用PBS洗涤3次, 每次5 min。在切片中加入荧光二抗(1:100), 置于37℃暗处, 避光30 min, 然后用PBS洗涤3次, 每次5 min, 在倒置荧光显微镜下拍照。使用IPP软件分析荧光强度信息。

### 1.8 统计学处理

使用Graphpad Prism 7.0软件对数据进行分析。数据的两两比较采用方差分析后进行单因素方差分析检验。数据以均数±标准差( $\bar{x} \pm s$ )表示。 $P < 0.05$ 为差异有统计学意义。

## 2 结果

### 2.1 奥曲肽对SAP大鼠肠道的保护作用

造模成功后SAP大鼠出现严重的小肠损伤, 肠壁充血、水肿、出血、炎性细胞浸润, 绒毛高度降低, 部分脱落和缺损, 黏膜上皮细胞变性坏死, 黏膜层和黏膜下层扩张, 提示奥曲肽治疗后明显改善肠道损伤(图1)。

### 2.2 奥曲肽对SAP大鼠血中炎症因子的调节作用

对SAP大鼠血中炎症因子的检测显示: 造模后SAP大鼠血清TNF- $\alpha$ , IL-1 $\beta$ 及MPO的含量显著上升; 而奥曲肽治疗组血清中TNF- $\alpha$ , IL-1 $\beta$ 及MPO的含量显著下降, 差异有统计学意义(均 $P < 0.001$ , 图2), 提示奥曲肽可调节机体免疫功能过度激活。

### 2.3 奥曲肽对SAP大鼠肠中炎症因子的调节作用

对各组大鼠的小肠组织中TNF- $\alpha$ , IL-1 $\beta$ 及MPO的水平进行了检测, 结果显示: SAP大鼠小肠组织中TNF- $\alpha$ , IL-1 $\beta$ 及MPO含量均显著高于对照组; 奥曲肽治疗能显著抑制SAP大鼠小肠组织中TNF- $\alpha$ , IL-1 $\beta$ 及MPO水平的升高, 差异有统计学意义(均 $P < 0.001$ , 图3), 表明奥曲肽对SAP大鼠肠道免疫微环境有保护作用。

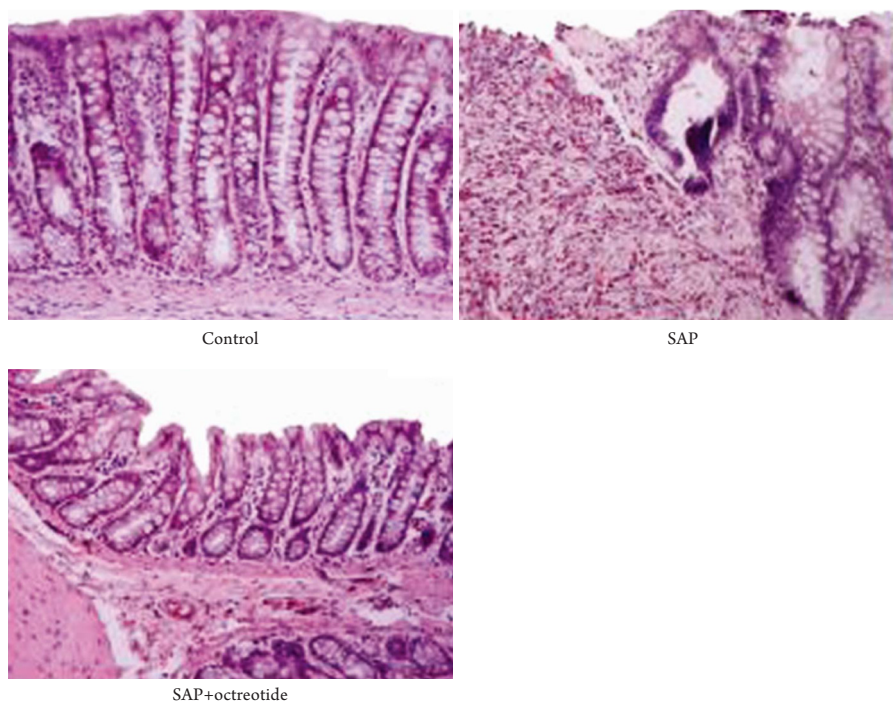


图1 奥曲肽对SAP大鼠肠道的保护作用(HE,  $\times 400$ )

Figure 1 Protective effect of octreotide on intestinal tract in rats with severe acute pancreatitis (HE,  $\times 400$ )

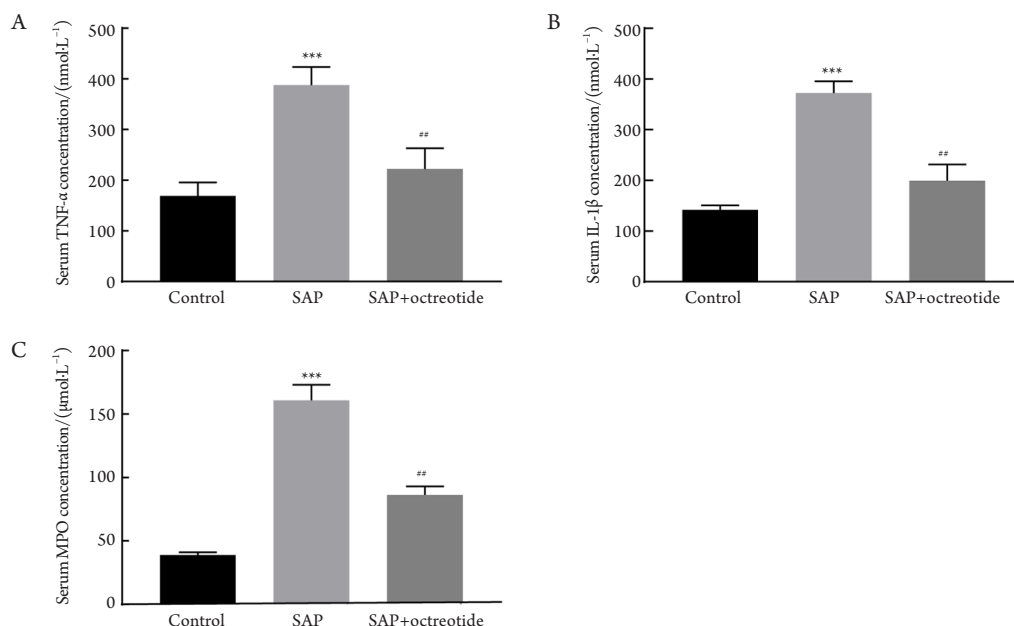


图2 奥曲肽对SAP大鼠血清中炎症因子的调节作用

**Figure 2 Effect of octreotide on the regulation of inflammatory factors in the serum of rats with severe acute pancreatitis**

(A)大鼠血清TNF- $\alpha$ 的含量；(B)大鼠血清IL-1 $\beta$ 的含量；(C)大鼠血清MPO的含量。与对照组相比，\*\*\* $P < 0.001$ ；与SAP组相比，\*\* $P < 0.01$ 。

(A) Level of TNF- $\alpha$  in serum of rats; (B) Level of IL-1 $\beta$  in serum of rats; (C) Level of MPO in serum of rats. Compared with Control group, \*\*\* $P < 0.001$ ; compared with SAP group, \*\* $P < 0.01$ .

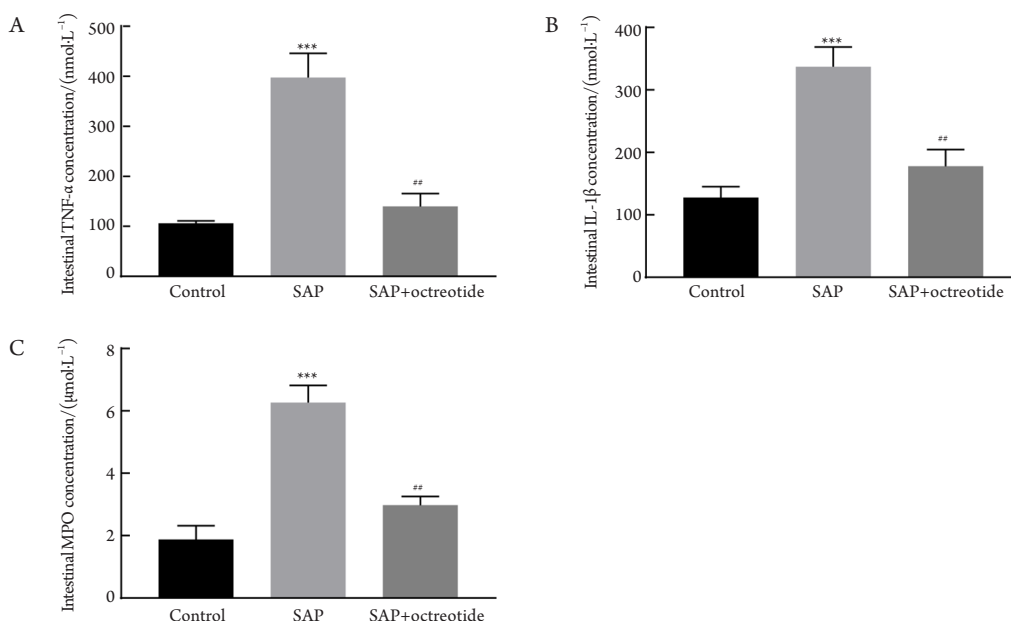


图3 奥曲肽对SAP大鼠肠组织中炎症因子的调节作用

**Figure 3 Effect of octreotide on the regulation of inflammatory factors in the intestinal tissue of rats with severe acute pancreatitis**

(A)大鼠肠组织中TNF- $\alpha$ 的含量；(B)大鼠肠组织中IL-1 $\beta$ 的含量；(C)大鼠肠组织中MPO的含量。与对照组相比，\*\*\* $P < 0.001$ ；与SAP组相比，\*\* $P < 0.01$ 。

(A) Level of TNF- $\alpha$  in intestinal tissue of rats; (B) Level of IL-1 $\beta$  in intestinal tissue of rats; (C) Level of MPO in intestinal tissue of rats. Compared with Control group, \*\*\* $P < 0.001$ ; compared with SAP group, \*\* $P < 0.01$ .

## 2.4 奥曲肽对 SAP 大鼠肠毛细血管通透性的调节作用

SAP大鼠伊文思蓝染色结果(图4A)显示:染料外渗明显增高, SAP大鼠肠组织湿干重比值(图4B)显著高于正常大鼠, 差异有统计学意义(均 $P<0.001$ ); 而奥曲肽治疗能显著抑制SAP大鼠血管蛋白外渗并使SAP组大鼠肠组织湿干重比明显降低, 差异有统计学意义( $P<0.001$ )。表明奥曲肽可减弱SAP大鼠肠道通透性升高, 对SAP所致肠黏膜损伤有保护作用。

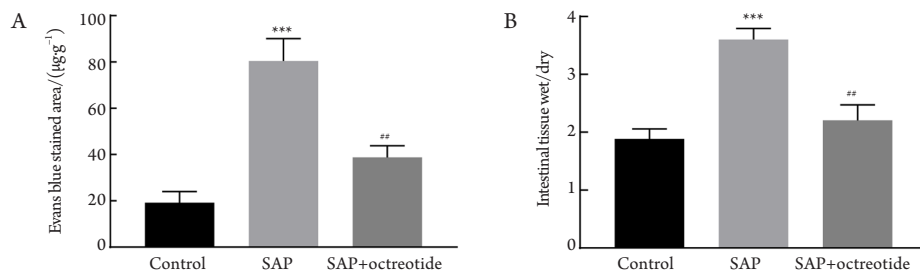


图4 奥曲肽对SAP大鼠肠毛细血管通透性的影响

### Figure 4 Effect of octreotide on intestinal capillary permeability in rats with severe acute pancreatitis

(A)大鼠肠组织中伊文思蓝染色比重; (B)大鼠肠组织中湿干重比值。与对照组相比,  $***P<0.001$ ; 与SAP组相比,  $**P<0.01$ 。(A) Proportion of Evans blue staining in intestinal tissue of rats; (B) Ratio of wet to dry weight in intestinal tissue of rats. Compared with Control group,  $***P<0.001$ ; compared with SAP group,  $**P<0.01$ .

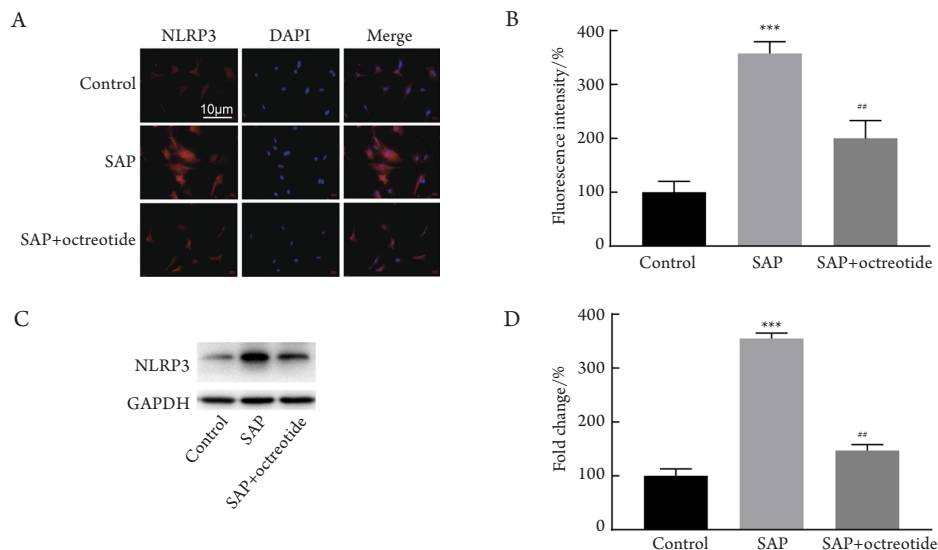


图5 奥曲肽对SAP大鼠肠组织中NLRP3表达的影响

### Figure 5 Effect of octreotide on the expression of NLRP3 in intestinal tissue of rats with severe acute pancreatitis

(A)大鼠肠组织中NLRP3免疫荧光染色; (B)大鼠肠组织中NLRP3染色结果统计图; (C)大鼠肠组织中NLRP3免疫荧光试验; (D)大鼠肠组织中NLRP3免疫印迹试验结果统计图。与对照组相比,  $***P<0.001$ ; 与SAP组相比,  $**P<0.01$ 。(A) NLRP3 immunofluorescence staining in rat intestinal tissue; (B) Statistical map of NLRP3 staining results in intestinal tissue of rats; (C) NLRP3 immunofluorescence test in rat intestinal tissue; (D) Statistical charts of NLRP3 immunoblotting results in rat intestinal tissues. Compared with Control group,  $***P<0.001$ ; compared with SAP group,  $**P<0.01$ .

### 3 讨论

SAP的发病凶险且伴随较高病死率,目前尚缺乏有效的治疗手段<sup>[3]</sup>。肠道免疫系统失调在SAP致病损伤中起重要作用。正常肠黏膜依赖屏障系统维持动态平衡,能有效防止肠道细菌的入侵,肠道免疫和屏障功能的损害可能导致肠黏膜屏障通透性的增加,从而影响肠功能<sup>[4-6]</sup>;细菌及其代谢产物因而进入肠淋巴管和血液系统,进一步诱导炎症介质的释放引起全身炎症<sup>[8]</sup>。本研究发现SAP大鼠肠黏膜组织中产生了严重的病理损伤,血清和肠组织中炎症因子的水平显著升高提示系统性及肠道局部炎症环境的出现。文献[9]中对各种药物疗效的检测时间为3~24 h,12 h后的疗效比较确切稳定,因此笔者选择在造模18 h后取样观测。伊文思蓝染色和湿干重比的结果进一步证明SAP大鼠肠屏障功严重受损。给予奥曲肽治疗后,SAP大鼠肠损伤和免疫应激水平显著降低。奥曲肽可降低内脏血流量,降低门脉系统血压,减少肠道分泌液过度分泌,增加肠道对水的吸收等功能,具有调节肠道免疫功能的作用一致<sup>[10-12]</sup>。本研究结果表明:奥曲肽对SAP所致的肠损伤有显著的保护作用,可降低SAP大鼠血液和肠中炎性因子的水平,降低微血管通透性和组织水肿,恢复肠道至免疫功能的正常,防止进一步肠道损伤。肠道免疫功能主要依赖于肠黏膜表面免疫细胞的非特异性和特异性免疫,先天免疫在SAP发展中的作用已受到广泛关注<sup>[13]</sup>。肠道中的天然免疫细胞可以通过模式识别受体激活炎症信号通路,迅速启动炎症反应<sup>[14]</sup>。NLRP3炎症体是最具特征的NLR家族成员之一,是天然免疫系统的关键组成部分<sup>[15]</sup>。本研究结果显示:SAP大鼠小肠中NLRP3表达显著高于正常大鼠,奥曲肽显著降低SAP大鼠小肠中NLRP3的表达,表明奥曲肽可通过降低NLRP3炎症小体,从而进一步减轻SAP中过量的先天免疫介导的炎症反应和肠损伤。

综上,奥曲肽可有效调节肠道特异性免疫应答,并发挥重要的器官保护作用,在未来SAP消化器官保护治疗中有望成为特效药物。

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