

蟛蜞菊内酯在重症急性胰腺炎肠屏障功能中的作用及机制

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摘要

目的: 探讨蟛蜞菊内酯(Wedelolactone)在重症急性胰腺炎(SAP)大鼠肠屏障损伤中的作用及机制。方法: 选取SPF级健康雄性Wistar大鼠40只, 随机分为4组: 假手术组(SO组)、重症急性胰腺炎组(SAP组)、蟛蜞菊内酯25 mg/kg治疗组(Wed-L)和蟛蜞菊内酯50 mg/kg治疗组(Wed-H)。采用逆行胆胰管穿刺法, 向胆胰管内注入5%牛黄胆酸钠溶液(1 ml/kg)诱导SAP模型。假手术组给予等量的生理盐水。Wed-L组和Wed-H组在SAP模型建立后在1小时和6小时后给予腹腔注射25和50mg/kg的蟛蜞菊内酯。制模24 h后, 再次麻醉大鼠。使用自动生化分析仪检测血液中的血清淀粉酶和脂肪酶水平及血清肿瘤坏死因子- α 、白细胞介素-6, 白细胞介素-18和白细胞介素-1 β 均使用标准诊断试剂盒进行检测; HE用于评估胰腺、肠道病理变化, 并进行病理评分; 通过用Western blot技术测定肠组织中细胞焦亡相关蛋白GSDMD、Caspase-11和紧密连接蛋白ZO-1、Occludin、Claudins-1的表达; RT-PCR检测细胞焦亡相关基因GSDMD和Caspase-11的表达情况。使用免疫荧光染色法测定肠上皮细胞细胞焦亡相关蛋白GSDMD、Caspase-11和紧密连接蛋白ZO-1、Occludin、Claudins-1的分布情况。结果: HE染色结果示, 与SO组相比, SAP组出现明显肠道病理损伤($P < 0.01$), 而Wed-H组肠道损伤明显低于SAP组($P < 0.01$); 同样, SAP组胰腺损伤明显高于SO组($P < 0.01$), Wed-H组的损伤较于SAP组的胰腺损伤减轻($P < 0.05$), Wed-L和Wed-H之间的差异无统计学意义。SAP组的血清淀粉酶和脂肪酶水平明显高于SO组($P < 0.01$), Wed-L和Wed-H组明显低于SAP组($P < 0.01$)。SAP组TNF- α 、IL-6、IL-1 β 、IL-18水平明显高于SO组($P < 0.01$), Wed-L和Wed-H组明显低于SAP组($P < 0.01$)。RT-PCR结果显示SAP组肠道组织GSDMD和Caspase-11的表达水平较SO组明显升高($P < 0.01$), Wed-H组的表达水平明显低于SAP组($P < 0.01$)。Western blot显示, SAP组肠道组织GSDMD和Caspase-11蛋白表达水平较SO组升高($P < 0.05$)。与SAP组相比较, Wed-H组肠道组织GSDMD和Caspase-11蛋白表达水平明显下降($P < 0.05$)。SAP组肠道组织ZO-1、Claudin-1、Occludin蛋白表达水平较SO组降低($P < 0.01$)。与SAP组相比较, Wed-H组肠道组织ZO-1、Claudin-1、Occludin蛋白表达水平明显升高($P < 0.01$)。免疫荧光结果显示SAP组GSDMD明显高于SO组和Wed-H组, 而紧密连接蛋白ZO-1、Occludin、Claudin-1显示较SO组弱, Wed-H组较SAP明显改善。结论: 蟛蜞菊内酯通过抑制Caspase-11下调GSDMD表达, 减少其介导的细胞焦亡炎性因子的释放, 减轻肠粘膜损伤。

关键词

重症急性胰腺炎, 肠屏障, 细胞焦亡, 蟛蜞菊内酯

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The Role and Mechanism of Wedelolactone in Intestinal Barrier Function of Severe Acute Pancreatitis

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Abstract

Objective: To explore the role and mechanism of Wedelolactone in intestinal barrier injury in rats with severe acute pancreatitis (SAP). **Methods:** Forty SPF healthy male Wistar rats were randomly divided into four groups: sham operation group (SO group), severe acute pancreatitis group (SAP group), wedelolactone 25 mg/kg treatment group (Wed-L) and wedelolactone 50 mg/kg treatment group (Wed-H). SAP model was induced by retrograde cholangiopancreatography by injecting 5% sodium taurocholate solution (1 ml/kg) into cholangiopancreatography. The sham operation group was given the same amount of normal saline. Wed-L group and Wed-H group were given intraperitoneal injection of 25 and 50 mg/kg wedelolactone 1 hour and 6 hours after the establishment of SAP model. After 24 hours of modeling, the rats were anesthetized again. The levels of serum amylase and lipase in blood and serum tumor necrosis factor- α , interleukin-6, interleukin-18 and interleukin-1 β were detected by automatic biochemical analyzer, and standard diagnostic kits were used. HE was used to evaluate the pathological changes of pancreas and intestine, and made pathological score. The expressions of apoptosis-related proteins GSDMD, Caspase-11 and tight junction proteins ZO-1, Occludin and Claudins-1 in intestinal tissues were determined by Western blot. RT-PCR was used to detect the expression of apoptosis-related genes GSDMD and Caspase-11. The distribution of apoptosis-related proteins GSDMD, Caspase-11 and tight junction proteins ZO-1, Occludin and Claudins-1 in intestinal epithelial cells was determined by immunofluorescence staining. **Results:** HE staining showed that compared with SO group, SAP group had obvious intestinal pathological injury ($P < 0.01$), while Wed-H group had significantly lower intestinal injury than SAP group ($P < 0.01$). Similarly, the pancreatic injury in SAP group was significantly higher than that in SO group ($P < 0.01$), and the pancreatic injury in Wed-H group was less than that in SAP group ($P < 0.05$). There was no significant difference between Wed-L and WED-H. Serum amylase and lipase levels in SAP group were significantly higher than those in SO group ($P < 0.01$), while those in Wed-L and Wed-H groups were significantly lower than those in SAP group ($P < 0.01$). The levels of TNF- α , IL-6, IL-1 β and IL-18 in SAP group were significantly higher than those in SO group ($P < 0.01$), while those in Wed-L and Wed-H groups were significantly lower than those in SAP group ($P < 0.01$). RT-PCR results showed that the expression levels of GSDMD and Caspase-11 in intestinal tissues in SAP group were significantly higher than those in SO group ($P < 0.01$), and the expression levels in Wed-H group were significantly lower than those in SAP group ($P < 0.01$). Western blot showed that the expression levels of GSDMD and Caspase-11 protein in intestinal tissues of SAP group were higher than those of SO group ($P < 0.05$). Compared with SAP group, the expression levels of GSDMD and Caspase-11 protein in intestinal tissue of Wed-H group decreased significantly ($P < 0.05$). The expression levels of ZO-1, Claudin-1 and Occludin in intes-

tinal tissues in SAP group were lower than those in SO group ($P < 0.01$). Compared with SAP group, the expression levels of ZO-1, Claudin-1 and Occludin in intestinal tissue in Wed-H group were significantly higher ($P < 0.01$). Immunofluorescence results showed that GSDMD in SAP group was significantly higher than that in SO group and Wed-H group, while tight junction proteins ZO-1, Occludin and Claudin-1 were weaker than that in SO group, and Wed-H group was significantly improved than that in SAP. Conclusion: Wedelolactone down-regulates the expression of GSDMD by inhibiting Caspase-11, reduces the release of inflammatory factors in pyroptosis mediated by Caspase-11, and reduces intestinal mucosal injury.

Keywords

Severe Acute Pancreatitis, Intestinal Barrier, Pyroptosis, Wedelolactone

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1. 引言

急性胰腺炎(acute pancreatitis, AP)是一种突发的胰腺炎症,是消化系统疾病住院的主要原因之一[1]。当患者出现持续性的器官衰竭,则发展为重症急性胰腺炎(severe acute pancreatitis, SAP),胰腺继发感染是最严重的相关并发症,是高死亡率的主要原因[2]。SAP时患者肠屏障功能受损,细菌移位进入全身循环,进而导致继发感染,发展至全身炎症反应,甚至多器官功能障碍[3]。螞蟥菊内酯(wedelolactone, Wed)是一种植物来源的香豆素,可以直接抑制 IKK 复合物来抑制 caspase-11 表达,具有抗肿瘤、抗骨质疏松、抗纤维化、抗丙型肝炎病毒和降低胆固醇等多种活性[4]。细胞焦亡是一种由炎性半胱天冬酶诱导的坏死和炎症程序性细胞死亡。细胞焦亡通过 caspase-1 依赖性或非依赖性机制来调节。Caspase-1 非依赖性细胞焦亡由人 caspase-4、caspase-5 或小鼠 caspase-11 执行,表现为细胞膜破裂,导致细胞的细胞质内容物释放,包括促炎细胞因子、内源性配体和其他危险相关分子模式[5]。有研究表明,感染性炎症患者肠道中广泛存在炎性半胱天冬酶 caspase-11 激活[6]。已有证明,细胞焦亡与许多炎症性疾病有关,如败血症,哮喘,脑出血等[7], Liu 等人通过实验证明抑制细胞焦亡改善了败血症诱发的心肌病[8]; Pan 等人通过实验证明抑制细胞焦亡可以减轻脑出血后的神经炎症反应[9]; Liu 等人通过研究发现抑制细胞焦亡减轻哮喘患者的中性粒细胞气道炎症[10]。然而非经典途径细胞焦亡在 SAP 肠屏障损伤中的作用尚未可知,因此,我们推测抑制 caspase-11 介导的细胞焦亡可以为 SAP 的治疗提供新的方向。本实验通过逆行穿刺胆管,向胆胰管内注入 5%牛磺胆酸钠构建大鼠 SAP 模型,检测肠道 GSDMD 和紧密连接蛋白的表达,并通过螞蟥菊内酯抑制 caspase-11 降低 GSDMD 的表达,从而探讨非经典途径细胞焦亡对 SAP 大鼠肠道功能的影响。

2. 材料与方法

2.1. 实验动物与分组

8 周龄 SPF 级雄性 Wistar 大鼠 40 只,体重 250~300 g,由北京斯贝福生物技术有限公司提供,青岛大学中心实验室饲养。将大鼠圈养在 12 小时光照/黑暗循环下, $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ 、和 45%~55%的湿度。动物护理和处理程序遵循实验动物护理和使用指南,并经青岛大学动物福利伦理委员会批准。按照随机数字表法将大鼠分为假手术组(SO),SAP 模型组, SAP Wed-H 组, SAP Wed-L 组,每组 10 只。

2.2. 主要实验试剂

牛磺胆酸钠购自美国 Sigma 公司, 蟾蜍菊内酯购自美国 MedChemexpress 公司, RNA 提取试剂购自美国 Thermo Fisher 公司, 反转录和荧光定量试剂盒购自日本 TaKaRa 公司, GSDMD 抗体、Caspase-11 抗体购自美国 Proteintech Group 公司, ZO-1 抗体、Occludin 抗体和 Claudin-1 抗体购自美国 Abcam 公司, 血清肿瘤坏死因子- α (TNF- α)、白细胞介素(interleukins, IL-6、IL-1 β 和 IL-18)酶联免疫吸附试验(enzyme linked immunosorbent assay, ELISA)检测试剂盒购自武汉 Boster 公司。

2.3. 动物模型制备

实验动物均于实验前禁食 12 h, 自由饮水。大鼠称重麻醉后夹住肝门部胰胆管, 将连接微量泵的 24 G 套管针逆行穿进胰胆管, 通过标准压力控制向胰胆管内注入 5% 牛磺胆酸钠(1 ml/kg)诱导 SAP 模型。假手术组的大鼠接受剖腹手术和相同体积的盐水溶液。Wedelolactone (SAP + Wed)治疗组的大鼠在手术后 1 小时和 6 小时通过腹膜内注射接受 25 mg/kg 或 50 mg/kg wedelolactone。

2.4. 检测指标及方法

各组于制膜后 24 小时麻醉后取下腔静脉血; 采取颈椎脱臼法处死大鼠, 收集胰腺、回肠组织于 4% 多聚甲醛溶液中固定, 用于组织学分析, 其余部分于-80 $^{\circ}$ C 保存备用。

1) 血清脂肪酶和淀粉酶: 使用自动分析仪测定血液样本中的血清淀粉酶(amylase, AMY)和脂肪酶(lipase, LIPA)的活性。

2) 胰腺病理学观察及病理评分: 取胰腺组织, 置于 4% 甲醛溶液中固定, 常规进行脱水、石蜡包埋、切片, 苏木素-伊红(hematoxylin-eosin, HE)染色, 中性树脂封片, 光镜下观察。采用双盲法, 按 Schmidt 等提出的胰腺组织病理评分标准评分[11]。

3) 肠道病理学观察及病理评分: 取回肠组织, 同上法 HE 染色, 200 倍光镜下观察组织切片。按照 Chiu 分级评分标准进行小肠组织病理评分[12]。

4) 酶联免疫吸附测定(ELISA)分析: 严格按照试剂盒说明书步骤操作, 通过相应试剂盒检测血清和细胞上清液中 TNF- α , IL-6, IL-1 β , IL-18 的水平。

5) 蛋白质免疫印迹实验(Western blott)检测肠组织 GSDMD、caspase-11 与 TJ 蛋白: 通过 RIPA 裂解缓冲液从回肠组织中分离蛋白质, 然后通过 BCA 蛋白质测定试剂盒测定蛋白质浓度。将蛋白质样品加入制备好的凝胶孔中进行电泳分离, 然后转移到聚偏氟乙烯膜上。用 5% 脱脂牛奶封闭细胞膜 1 h, 并在 4 $^{\circ}$ C 与一抗孵育过夜, 最后在室温下与相应二抗孵育 1 h, 进行条带显影。应用 Image-J 软件对 Western blot 条带的密度进行定量。

6) 反转录 - 聚合酶链反应(RT-PCR)检测肠道 GSDMD 和 caspase-11 的表达: 通过使用 TRIzol 试剂提取总 RNA, 将提取的 RNA 用紫外分光光度计进行检测, 测量其在 260 nm/280 nm 波长处 OD 值, 判断纯度。按照 TaKaRa 试剂盒要求进行反转录合成 cDNA, 以 β -肌动蛋白(β -actin)为内参, 按照 $2^{-\Delta\Delta Ct}$ 法计算 GSDMD 和 caspase-11 的表达量。

7) 免疫荧光法测定 TJ 蛋白和 GSDMD 表达: 取回肠组织, 石蜡包埋、切片, 烘干、水化, 抗原热修复, 用山羊血清封闭, 加入一抗 4 $^{\circ}$ C 孵育过夜, 二抗孵育, DAPI 核染色, 滴加抗荧光淬灭剂封片, 荧光显微镜下观察并拍照。

2.5. 统计学方法

数据表示为平均值 \pm SD。使用 GraphPad Prism 软件版本 8.0 (GraphPad, San Diego, CA, USA), 使用

单因素方差分析加 Tukey 多重比较检验或 Kruskal-Wallis 非参数检验分析组间差异。 $P < 0.05$ 定义为差异具有统计学意义。

3. 结果

3.1. 胰腺和肠道病理学改变

光镜下显示(见图 1), SO 组大鼠胰腺(A)和肠黏膜(E)均未见明显损伤。SAP 组(B)和 Wed-H 组(D)大鼠胰腺均有不同程度的水肿、出血及炎性细胞浸润, 但 Wed-H 组病理学改变较 SAP 模型组明显减轻。SAP 组(F)和 Wed-H 组(H)大鼠回肠组织也可见不同程度的损伤, Wed-H 组损伤程度较 SAP 组有所减轻。SAP 组大鼠肠黏膜内可见绒毛破损, 部分可见局部出血, 炎性细胞浸润, 间质水肿; Wed-H 组大鼠肠壁绒毛上皮间下间隙增大, 伴血管淤血, 炎性细胞浸润, 肠壁水肿。

定量分析显示(见图 2): SAP 组和 Wed-H 组大鼠胰腺组织与肠黏膜病理评分均明显高于 SO 组($P < 0.01$), 但 Wed-H 组病理评分明显低于 SAP 组($P < 0.01$)。Wed-L 和 SAP 组之间损伤无统计学意义($P > 0.05$), Wed-H 和 Wed-L 之间损伤无明显差异($P > 0.05$)。

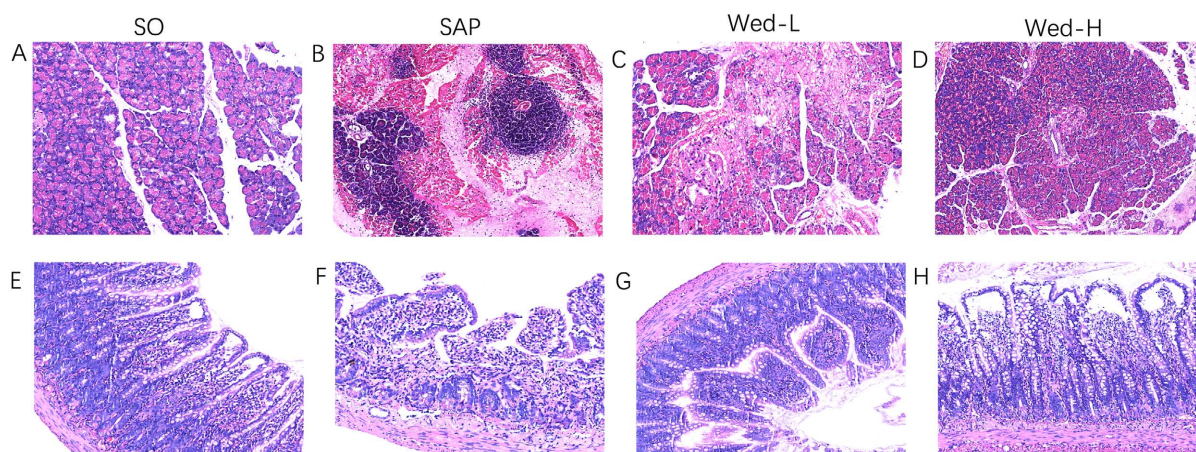


Figure 1. Pathological changes of pancreas and small intestine of rats in each group ($\times 400$ times). Normal pancreatic cells and normal intestinal epithelial cells can be seen in SO group. In SAP group, diffuse edema, interlobular space widening, inflammatory cell infiltration and partial parenchymal hemorrhage were observed. It is obvious that intestinal villi rupture and inflammatory cell infiltration in the intestine. The number of pancreatitis cells in Wed-H group was lower than that in SAP group. The epithelial-subcutaneous space of intestinal villi was enlarged, with partial edema and inflammatory cell infiltration, but the degree was less than that of SAP model group

图 1. 各组大鼠胰腺、小肠 HE 染色病理情况($\times 400$ 倍)。SO 组可见正常胰腺细胞和正常肠道上皮细胞。SAP 组可见弥漫性水肿, 小叶间隙增宽, 炎性细胞浸润, 部分实质出血; 肠道绒毛断裂, 炎性细胞浸润。Wed-H 组胰腺炎性细胞数量较 SAP 组减少; 肠道肠壁绒毛上皮间下间隙增大, 部分水肿, 炎性细胞浸润, 但程度较 SAP 模型组减轻

3.2. 肠道组织 GSDMD 及 Caspase-11 表达(见图 3)

通过 PCR 检测细胞焦亡非经典途径 GSDMD 和 Caspase-11 的表达, 结果分析显示与 SO 组相比, SAP 回肠组织中细胞焦亡相关基因 GSDMD 和 Caspase-11 的 mRNA 水平明显升高($P < 0.05$); 相比于 SAP 组, Wed-H 组上述指标明显降低($P < 0.05$)。Wed-L 组和 SAP 组之间差异无统计学意义。

3.3. 肠道组织 TJ 蛋白和细胞焦亡蛋白表达(见图 4)

用免疫印迹法检测各组大鼠回肠 GSDMD、Caspase-11 和 TJ 蛋白的表达。与 SO 组相比, SAP 组的 ZO-1、occludin 和 claudin-1 的表达显著降低($P < 0.01$), GSDMD、Caspase-11 的表达显著升高($P < 0.01$);

与 SAP 组相比, Wed-H 组的 ZO-1、occludin 和 claudin-1 的表达显著升高($P < 0.01$), GSDMD、Caspase-11 的表达显著降低($P < 0.01$)。

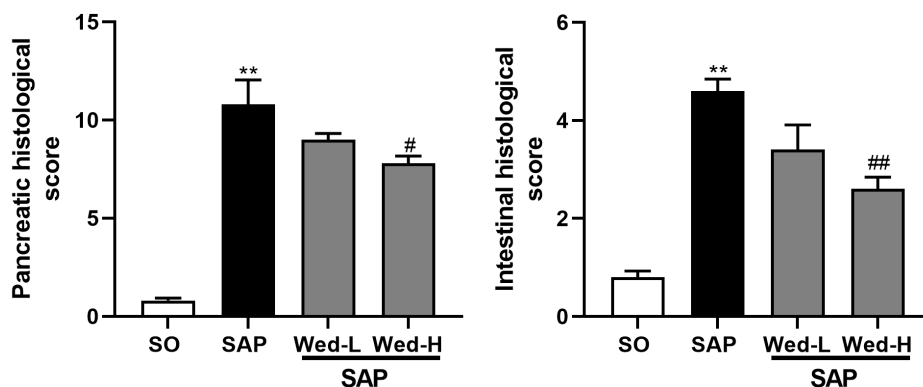


Figure 2. Quantitative analysis of pathological scores of pancreatic and intestinal tissues. Note: **, $P < 0.01$ compared with SO group; ##, compared with SAP group, $P < 0.01$; #, compared with SAP group, $P < 0.05$

图 2. 胰腺、肠道组织病理评分定量分析。注: **, 与 SO 组相比 $P < 0.01$; ##, 与 SAP 组相比 $P < 0.01$; #, 与 SAP 组相比 $P < 0.05$

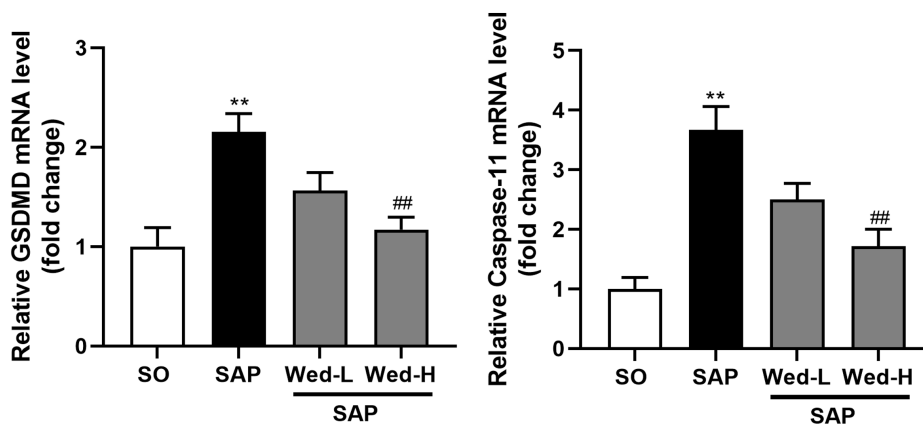


Figure 3. Relative quantitative analysis of GSDMD and Caspase-11. Note: **, $P < 0.01$ compared with SO group; ##, $P < 0.01$ compared with SAP group

图 3. GSDMD、Caspase-11 相对定量分析。注: **, 与 SO 组相比 $P < 0.01$; ##, 与 SAP 组相比 $P < 0.01$

3.4. 细胞因子水平(见图 5)

全自动生化仪检测脂肪酶和淀粉酶示 SAP 组、Wed-H 和 Wed-L 组血清脂肪酶和淀粉酶水平明显高于 SO 组。酶联免疫吸附法检测血清中 TNF- α 、IL-6、IL-18 和 IL-1 β 浓度, 并对其进行定量分析, 结果显示为在 SAP 组大鼠中 TNF- α 、IL-6、IL-1 β 、IL-18 浓度较 SO 组上升($P < 0.05$), Wed-H 和 Wed-L 组 TNF- α 、IL-6、IL-1 β 、IL-18 浓度较 SAP 组下降($P < 0.05$)。

3.5. 肠道组织 TJ 蛋白和细胞焦亡蛋白的分布(见图 6)

通过免疫荧光检测 GSDMD 和 TJ 蛋白在肠道中的表达, 结果显示肠上皮各细胞之间检测到 GSDMD (红色)、Claudin-1 (绿色)、ZO-1 (绿色)和 Occludin (绿色)。SAP 组有大量 GSDMD (红色)聚集, 表明细胞焦亡被激活。Wed-H 组 GSDMD 浓度明显低于 SAP 组。SAP 组 Claudin-1、ZO-1 和 Occludin 明显低于 SO 组。Wed-H 组 Claudin-1、ZO-1 和 Occludin 较 SAP 组明显改善。

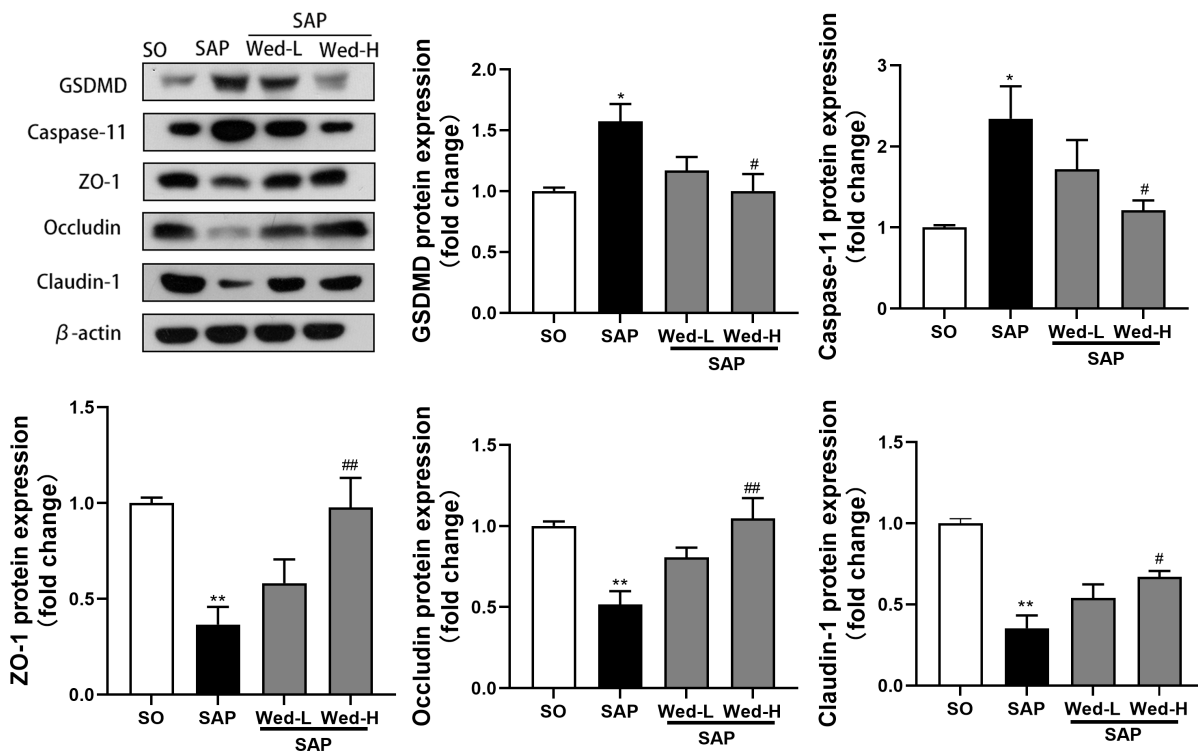


Figure 4. Results and relative quantitative analysis of intestinal TJ and cytokeratin. Note: **, $P < 0.01$ compared with SO group; ##, compared with SAP group, $P < 0.01$; #, compared with SAP group, $P < 0.05$

图 4. 肠道 TJ 及细胞焦亡蛋白结果及相对定量分析。注: **, 与 SO 组相比 $P < 0.01$; ##, 与 SAP 组相比 $P < 0.01$; #, 与 SAP 组相比 $P < 0.05$

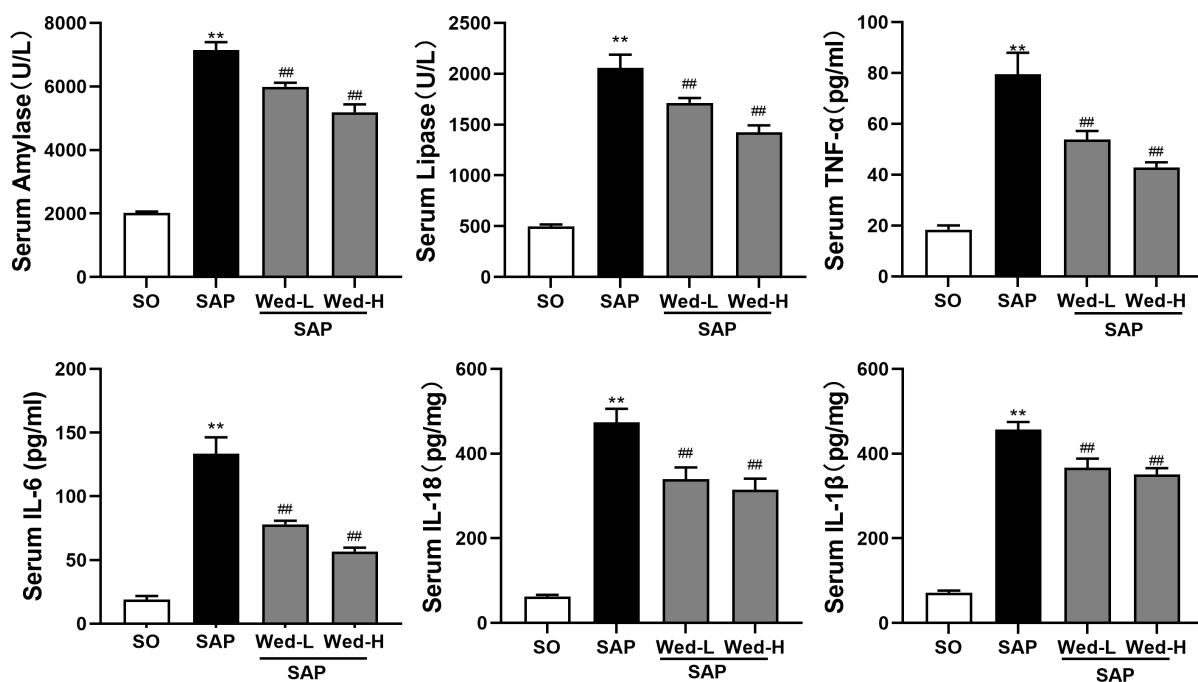


Figure 5. Relative quantitative analysis of cytokine levels. Note: **, $P < 0.01$ compared with SO group; ##, $P < 0.01$ compared with SAP group

图 5. 细胞因子水平相对定量分析。注: **, 与 SO 组相比 $P < 0.01$; ##, 与 SAP 组相比 $P < 0.01$

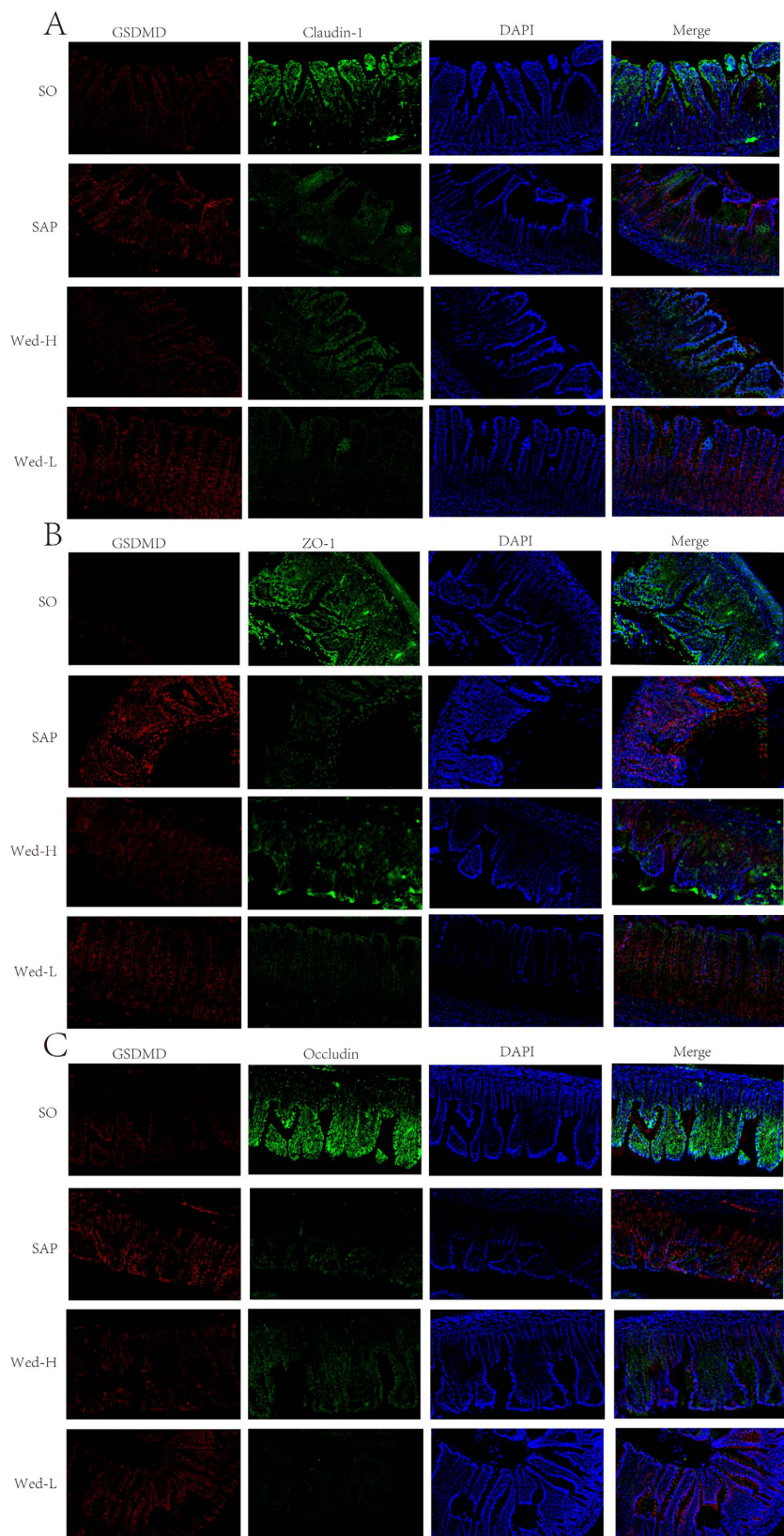


Figure 6. Distribution of GSDMD and TJ in intestinal epithelial cells of rats in each group ($\times 200$ times)
图 6. 各组大鼠肠上皮细胞 GSDMD 和 TJ 的分布($\times 200$ 倍)

4. 讨论

重症急性胰腺炎是一种高死亡率、发展迅速的疾病,其发病机制涉及一系列因素,尚未完全阐明[13]。SAP 肠屏障功能受损,细菌移位进入全身循环,进而导致继发感染,发展至全身炎症反应,甚至多器官功能障碍,从而导致一系列并发症[14]。最近的研究表明,肠上皮细胞中存在着经典途径的细胞焦亡[15],因此,我们通过 SAP 大鼠模型探讨非经典途径细胞焦亡即 caspase-11 在肠屏障功能中的作用。本研究观察了大鼠血清炎症指标水平及回肠组织形态学变化,表明 caspase-11 依赖性的细胞焦亡参与 SAP 的发展过程,其肠屏障功能明显受损。此外,本研究观察到通过螯螬菊内酯抑制 caspases-11 依赖性的细胞焦亡可以使以上损伤得到改善。

肠屏障是一种独特的粘膜屏障,既能消化和吸收营养,又能防御有害物质,如肠道有毒物质、病原体 and 抗原[16]。肠屏障由四部分组成:机械屏障、免疫屏障、化学屏障和生物屏障。完整的肠屏障在维持人体健康和治疗胃肠疾病甚至肠外疾病中起重要作用[17]。紧密连接,一种细胞连接,也是机械屏障的重要组成部分。TJ 是由 TJ 蛋白复合物形成的高度动态结构,位于内皮细胞细胞间隙的外侧。TJ 包括多种蛋白质, claudin 和 occludin 是跨膜蛋白, ZO 是细胞质中的衔接蛋白,它们的直接结合参与肠粘膜屏障完整性的调节[18]。本研究中通过免疫荧光检测 ZO-1、Claudin-1 和 Occludin 在肠道上皮的分布和通过免疫印迹检测肠道组织中 TJ 蛋白的含量来评估肠粘膜屏障功能,发现 SAP 大鼠 ZO-1、Claudin-1 和 Occludin 的表达下降,表明肠屏障功能受损。

细胞焦亡是一种程序性细胞死亡,然而其形态学不同于其他类型的程序性细胞死亡,如凋亡[19]。细胞凋亡有两张途径:外源性和内源性途径。每一种途径都需要特定的触发信号来启动能量依赖的分子事件级联,激活区自身的起始 caspases (8, 9, 10),进而激活执行 caspase-3。典型的凋亡细胞形态学包括细胞皱缩、染色质浓缩、脂质泡和凋亡小体[20]。与焦亡细胞相反,凋亡细胞保持着完整的细胞膜,细胞核受损而质膜保持完整[21]。细胞焦亡作为体内一种自然免疫反应,实际上更像是一把双刃剑。一方面,激活的炎症反应将病原体从宿主体内清除,并将感染的细胞转移到中性粒细胞和其他免疫细胞。由于炎症因子从其中释放,可以达到消灭感染性细菌和细胞内病原体的目的。另一方面,炎症反应的显著增加,不可避免地会导致致命损伤,如正常细胞死亡、组织损伤甚至器官衰竭等。当被脂多糖激活时, caspase-11 可以诱导非经典途径细胞焦亡。活化的 caspase-11 催化 GSDMD 裂解成 GSDMD-N 并穿透细胞膜,导致细胞焦亡,促进 IL-18 和 IL-1 β 的释放[19]。本实验中观察到 SAP 大鼠肠道组织中 caspase-11 和 GSDMD 蛋白显著增加,以及 IL-18 和 IL-1 β 水平的显著升高。总之,以上观察结果表明,在 SAP 肠屏障损伤中发生了 caspase-11 介导的细胞焦亡。

有研究证明, Wed 通过 GPX4 介导抑制细胞焦亡减轻急性胰腺炎相关肺损伤[22]。因此本实验中通过给予 Wed, 以确定抑制细胞焦亡能否改善 SAP 肠屏障损伤,结果发现 SAP 组 GSDMD 表达上升,而使用 Wed 可以降低 GSDMD 的表达并下降 IL-1 β 和 IL-18 含量,提高了 TJ 蛋白的表达,结合观察到的免疫荧光结果,表明抑制细胞焦亡后, SAP 大鼠炎症反应及程度明显降低,有效改善肠屏障损伤。

5. 结论

总之,在 SAP 肠屏障损伤大鼠中出现 caspase-11 依赖性细胞焦亡,通过 Wed 抑制 caspase-11 依赖性细胞焦亡可以减轻 SAP 肠屏障损伤并保护肠屏障功能。因此,靶向 caspase-11 依赖性细胞焦亡可能为治疗 SAP 肠损伤提供一种新的治疗靶点。

参考文献

- [1] Xiao, A.Y., Tan, M.L.Y., Wu, L.M., *et al.* (2016) Global Incidence and Mortality of Pancreatic Diseases: A Systematic

- Review, Meta-Analysis, and Meta-Regression of Population-Based Cohort Studies. *The Lancet Gastroenterology & Hepatology*, **1**, 45-55. [https://doi.org/10.1016/S2468-1253\(16\)30004-8](https://doi.org/10.1016/S2468-1253(16)30004-8)
- [2] Leppäniemi, A., Tolonen, M., Tarasconi, A., *et al.* (2019) WSES Guidelines for the Management of Severe Acute Pancreatitis. *World Journal of Emergency Surgery*, **14**, Article Number: 27. <https://doi.org/10.1186/s13017-019-0247-0>
- [3] Gui, M., Zhao, B., Huang, J., *et al.* (2023) Pathogenesis and Therapy of Coagulation Disorders in Severe Acute Pancreatitis. *Journal of Inflammation Research*, **16**, 57-67. <https://doi.org/10.2147/JIR.S388216>
- [4] Kabori, M., Yang, Z., Gong, D., *et al.* (2003) Wedelolactone Suppresses LPS-Induced Caspase-11 Expression by Directly Inhibiting the IKK Complex. *Cell Death & Differentiation*, **11**, 123-130. <https://doi.org/10.1038/sj.cdd.4401325>
- [5] Wright, S.S., Vasudevan, S.O. and Rathinam, V.A. (2022) Mechanisms and Consequences of Noncanonical Inflammation-Mediated Pyroptosis. *Journal of Molecular Biology*, **434**, Article ID: 167245. <https://doi.org/10.1016/j.jmb.2021.167245>
- [6] Man, S.M., Karki, R. and Kanneganti, T.D. (2017) Molecular Mechanisms and Functions of Pyroptosis, Inflammatory Caspases and Inflammasomes in Infectious Diseases. *Immunological Reviews*, **277**, 61-75. <https://doi.org/10.1111/immr.12534>
- [7] Yu, P., Zhang, X., Liu, N., *et al.* (2021) Pyroptosis: Mechanisms and Diseases. *Signal Transduction and Targeted Therapy*, **6**, Article Number: 128. <https://doi.org/10.1038/s41392-021-00507-5>
- [8] Liu, G., Chen, S., Yuan, X., *et al.* (2023) Mesencephalic Astrocyte-Derived Neurotrophic Factor (MANF) Alleviates Sepsis-Induced Cardiomyopathy by Inhibiting Pyroptosis. *Iranian Journal of Public Health*, **52**, 2380-2389. <https://doi.org/10.18502/ijph.v52i11.14052>
- [9] Lei, P., Li, Z., Hua, Q., *et al.* (2023) Ursolic Acid Alleviates Neuroinflammation after Intracerebral Hemorrhage by Mediating Microglial Pyroptosis via the NF- κ B/NLRP3/GSDMD Pathway. *International Journal of Molecular Sciences*, **24**, Article ID: 14771. <https://doi.org/10.3390/ijms241914771>
- [10] Liu, L., Zhou, L., Wang, L., *et al.* (2023) MUC1 Attenuates Neutrophilic Airway Inflammation in Asthma by Reducing NLRP3 Inflammasome-Mediated Pyroptosis through the Inhibition of the TLR4/MyD88/NF- κ B Pathway. *Respiratory Research*, **24**, Article Number: 255. <https://doi.org/10.1186/s12931-023-02550-y>
- [11] Schmidt, J.A.N., Rattner, D.W., Lewandrowski, K., *et al.* (1992) A Better Model of Acute Pancreatitis for Evaluating Therapy. *Annals of Surgery*, **215**, 44-56. <https://doi.org/10.1097/0000658-199201000-00007>
- [12] Chiu, C.-J., Mearle, A.H., Brown, R., *et al.* (1970) Intestinal Mucosal Lesion in Low-Flow States: I. A Morphological, Hemodynamic, and Metabolic Reappraisal. *Archives of Surgery*, **101**, 478-483. <https://doi.org/10.1001/archsurg.1970.01340280030009>
- [13] Banks, P.A., Bollen, T.L., Dervenis, C., *et al.* (2013) Classification of Acute Pancreatitis—2012: Revision of the Atlanta Classification and Definitions by International Consensus. *Gut*, **62**, 102-111. <https://doi.org/10.1136/gutjnl-2012-302779>
- [14] Wilden, A., Glaubitz, J., Otto, O., *et al.* (2022) Mobilization of CD11b+/Ly6chi Monocytes Causes Multi Organ Dysfunction Syndrome in Acute Pancreatitis. *Frontiers in Immunology*, **13**, Article ID: 991295. <https://doi.org/10.3389/fimmu.2022.991295>
- [15] Lee, S.-J., Jung, Y.H., Song, E.J., *et al.* (2015) *Vibrio vulnificus* VvpE Stimulates IL-1 β Production by the Hypomethylation of the IL-1 β Promoter and NF- κ B Activation via Lipid Raft-Dependent ANXA2 Recruitment and Reactive Oxygen Species Signaling in Intestinal Epithelial Cells. *The Journal of Immunology*, **195**, 2282-2293. <https://doi.org/10.4049/jimmunol.1500951>
- [16] Thoo, L., Noti, M. and Krebs, P. (2019) Keep Calm: The Intestinal Barrier at the Interface of Peace and War. *Cell Death & Disease*, **10**, Article Number: 849. <https://doi.org/10.1038/s41419-019-2086-z>
- [17] Lechuga, S. and Ivanov, A.I. (2017) Disruption of the Epithelial Barrier during Intestinal Inflammation: Quest for New Molecules and Mechanisms. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, **1864**, 1183-1194. <https://doi.org/10.1016/j.bbamcr.2017.03.007>
- [18] Slifer, Z.M. and Blikslager, A.T. (2020) The Integral Role of Tight Junction Proteins in the Repair of Injured Intestinal Epithelium. *International Journal of Molecular Sciences*, **21**, 972. <https://doi.org/10.3390/ijms21030972>
- [19] Shi, J., Zhao, Y., Wang, K., *et al.* (2015) Cleavage of GSDMD by Inflammatory Caspases Determines Pyroptotic Cell Death. *Nature*, **526**, 660-665. <https://doi.org/10.1038/nature15514>
- [20] Elmore, S. (2007) Apoptosis: A Review of Programmed Cell Death. *Toxicologic Pathology*, **35**, 495-516. <https://doi.org/10.1080/01926230701320337>
- [21] Chen, M., Rong, R. and Xia, X. (2022) Spotlight on Pyroptosis: Role in Pathogenesis and Therapeutic Potential of Ocular Diseases. *Journal of Neuroinflammation*, **19**, Article Number: 183. <https://doi.org/10.1186/s12974-022-02547-2>
- [22] Fan, R., Sui, J., Dong, X., *et al.* (2021) Wedelolactone Alleviates Acute Pancreatitis and Associated Lung Injury via GPX4 Mediated Suppression of Pyroptosis and Ferroptosis. *Free Radical Biology and Medicine*, **173**, 29-40. <https://doi.org/10.1016/j.freeradbiomed.2021.07.009>