珠子草素通过调控p38/JNK信号通路抑制肠上皮细胞凋亡保护肠屏障改善克罗恩病样肠炎

陶 露[1,2],陈 悦[2],黄林林[2],郑 旺[2],宋 雪[1,2],项 平[1,2],胡建国[2]
[1]蚌埠医科大学第一附属医院中心实验室,安徽 蚌埠 233004;[2]炎症相关性疾病基础与转化研究安徽省重点实验室,安徽 蚌埠 233004

摘要:目的 探讨天然化合物珠子草素(NIR)对克罗恩病样结肠炎的作用及其分子机制。**方法** 采用2,4,6-三硝基苯磺酸(TNBS)诱导小鼠建立结肠炎模型,随机分为4组:WT组注射生理盐水;WT+NIR组腹腔注射NIR(10 mg/kg,1次/d,注射7 d),TNBS组用2.5% TNBS造模并给予等体积的生理盐水;TNBS+NIR组用2.5% TNBS造模并腹腔注射NIR(10 mg/kg,1次/d,注射7 d),6只/组。用体质量变化、疾病活动指数(DAI)和结肠长度评估NIR的治疗效果。ELISA法和实时定量PCR(qRT-PCR)检测肠黏膜组织炎症因子(IL-6、IL-1β、TNF-α、IL-17A和IL-10)水平。TUNEL染色和Western blotting检测肠上皮细胞凋亡情况及相关蛋白(Bcl-2/Bax)的表达。Western blotting评估紧密连接蛋白(TJ)(ZO-1、Claudin-1)和p38/JNK通路的活化水平,并通过Diprovocim干预实验验证NIR的调控分子机制。**结果** NIR干预后TNBS小鼠体质量增加,DAI和组织学炎症评分减低,结肠长度增加($P<0.05$);ELISA和qRT-PCR结果表明NIR可降低促炎因子(IL-6,IL-1β、IL-17A和TNF-α)的蛋白和mRNA水平,上调抗炎因子IL-10表达水平($P<0.05$);TUNEL和Western blotting检测显示NIR可抑制肠上皮细胞凋亡,激活抗凋亡通路($P<0.05$);Western blotting结果证实NIR可上调ZO-1和Claudin-1的表达水平,并下调p38和JNK的磷酸化水平($P<0.05$);Diprovocim干预可衰减NIR对p38/JNK通路的失活作用。**结论** NIR可通过调控p38/JNK信号的活化抑制肠上皮细胞凋亡,从而改善小鼠CD样肠炎。

关键词:克罗恩病;炎症性肠病;肠上皮细胞凋亡;p38/JNK;珠子草素

Niranthin ameliorates Crohn's disease-like enteritis in mice by inhibiting intestinal epithelial cell apoptosis and protecting intestinal barrier *via* modulating p38/JNK signaling

TAO Lu[1,2], CHEN Yue[2], HUANG Linlin[2], ZHENG Wang[2], SONG Xue[1,2], XIANG Ping[1,2], HU Jianguo[2]
[1]Central Laboratory, First Affiliated Hospital of Bengbu Medical College, Bengbu 233004, China; [2]Anhui Provincial Key Laboratory of Basic and Translational Research on Inflammation-Related Diseases, Bengbu 233004, China

Abstract: Objective To investigate the therapeutic effect of the natural compound niranthin on Crohn's disease-like colitis in mice and explore the underlying molecular mechanisms. **Methods** In a mouse model of colitis induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS), the therapeutic effect of niranthin was evaluated by observing the changes in body weight, disease activity index (DAI), and colon length of the mice. The levels of inflammatory cytokines (IL-6, IL-1β, TNF-α, IL-17A and IL-10) in the intestinal mucosal tissue were detected using ELISA and quantitative real-time PCR (qRT-PCR). TUNEL staining and Western blotting were used to assess intestinal epithelial cell apoptosis and the expressions of Bcl-2 and Bax. The expression levels of tight junction proteins (ZO-1 and claudin-1) and the activation of the p38/JNK signaling pathway were investigated using Western blotting, and diprovocim intervention experiments were conducted to explore the molecular regulatory mechanism of niranthin. **Results** Niranthin treatment significantly increased body weight of TNBS-treated mice, lowered the DAI and histological inflammation scores, and increased colon length of the mice. The niranthin-treated mouse models showed obviously reduced protein and mRNA levels of IL-6, IL-1β, IL-17A, and TNF-α and upregulated expression of IL-10 in the colon tissue. TUNEL staining and Western blotting demonstrated that niranthin significantly inhibited intestinal epithelial cell apoptosis and activated the anti-apoptotic pathway in the mouse models. Niranthin treatment obviously upregulated the expression levels of ZO-1 and claudin-1 and downregulated the phosphorylation levels of p38 and JNK in the colon tissues of the mice. Diprovocim intervention obviously attenuated the inactivation of the p38/JNK signaling pathway induced by niranthin in the mouse models. **Conclusion** Niranthin ameliorates TNBS-induced Crohn's disease-like colitis in mice by inhibiting intestinal epithelial cell apoptosis and protecting the integrity of the intestinal barrier *via* regulating the activation of the p38/JNK signaling pathway.

Keywords: Crohn's disease; inflammatory bowel disease; intestinal epithelial cell apoptosis; p38/JNK; Niranthin

收稿日期:2025-04-28
基金项目:安徽省卫生健康委科研项目(AHWJ2024Aa40007);安徽省临床医学研究转化专项(202427b10020093);安徽省重点实验室开放课题(YZ2024D06);安徽省高校科学研究项目(2023AH040289)
作者简介:陶 露,硕士,研究实习员,E-mail: bytaolu@bbmu.edu.cn
通信作者:胡建国,博士,教授,E-mail: jghu9200@bbmu.edu.cn

克罗恩病(CD)是一种以慢性透壁性炎症为特征的炎症性肠病(IBD),病变多累及回肠末端及结肠,临床表现为反复腹痛、腹泻、体质量减轻及肠外并发症,严重影响患者生存质量[1-3]。目前研究认为,肠屏障功能障碍是CD的核心发病机制,肠上皮细胞异常凋亡可导致屏

障完整性破坏，促使肠道菌群易位至固有层并触发炎症反应[4-7]。现有治疗药物如氨基水杨酸制剂、糖皮质激素及免疫调节剂虽可缓解症状[8-11]，但存在疗效个体差异大、长期应用易导致耐药性及不良反应等问题，亟需开发新型治疗策略。珠子草素（NIR）是从毛茛属植物分离出来的木脂素，近年研究发现其具有抗炎、抗凋亡及抗肿瘤等多种生物活性[12-14]，但现有文献多聚焦于肿瘤或普通炎症模型，缺乏针对CD样肠炎"肠屏障损伤-慢性炎症"恶性循环的系统研究。值得注意的是，该化合物可通过抑制PI3K/Akt信号通路活化，降低TNF-α、IL-1β等促炎因子表达，同时增强细胞抗凋亡能力[14, 15]，提示其在调节肠道炎症及屏障修复中具有潜在的研究价值。然而，目前尚未见NIR干预CD样肠炎的相关报道，其具体作用靶点及分子机制仍有待阐明。本研究首次将NIR引入CD样肠炎研究体系，创新性提出"通过木脂素类化合物调控肠上皮细胞命运（抑制异常凋亡）重塑肠道屏障"的治疗策略，通过构建TNBS诱导小鼠结肠炎模型及LPS刺激Caco-2细胞模型，探讨其调控肠屏障功能的具体作用机制，揭示NIR改善CD样肠炎的潜在作用，为拓展IBD治疗药物研发提供新的实验依据。

1 材料和方法

1.1 主要材料

珠子草素（陶术生物）；苏木素伊红（HE）染色试剂盒和TUNEL染色试剂盒（索莱宝）；PrimeScript™ RT reagent Kit with gDNA Eraser（Perfect Real Time）试剂盒和TB Green™ Premix Ex Taq™ II（TII RNaseH Plus）试剂盒（Takara）；TNF-α、IL-1β、IL-6、IL-17A、IL-10和GAPDH引物（生工）；TNF-α、IL-1β、IL-6、IL-17A和IL-10 ELISA试剂盒（博士德）；Anti-ZO-1（1∶2000，Invitrogen）；Anti-Bcl-2（1∶2000）、Anti-Bax（1∶2000）、Anti-p38（1∶2000）、Anti-p-p38（1∶2000）、Anti-Claudin-1（1∶2000）、Anti-p-JNK（1∶2000）和山羊抗兔/鼠IgG H&L（Alexa Fluor® 555）（1∶1000）（Abcam）；Anti-GAPDH（1∶3000）和HRP标记山羊抗兔/鼠IgG（1∶3000）（中杉金桥）；Diprovocim（MCE）。

1.2 动物分组

选取24只体质量20~25 g的C57BL/6J雌性小鼠，随机分为4组：WT组，WT+NIR组，TNBS组和TNBS+NIR组，6只/组。TNBS造模[16]：将无水乙醇与5% TNBS药剂按1∶1体积比混合配置2.5% TNBS药剂；实验前1 d禁食，取1 mL注射器连接10号灌肠针后抽取约0.1 mL 2.5%TNBS溶液；待用石蜡油涂抹镊子并润滑肛门后用镊子扩肛；将灌肠针缓慢插入至最深处，用针栓连接处堵住肛门，通过旋转注射器活塞柄将药液打入小鼠肠管内，注入完成后再缓慢轻柔的退出灌肠针。WT组注射生理盐水；WT+NIR组腹腔注射NIR（10 mg/kg[14]，1次/d，注射7 d），TNBS组用2.5% TNBS造模并给予等体积的生理盐水；TNBS+NIR组用2.5% TNBS造模并腹腔注射NIR（10 mg/kg[14]，1次/d，注射7 d），第8天小鼠脱颈处死并取检。C57BL/6J小鼠购自江苏集萃药康生物科技股份有限公司，饲养在SPF级动物中心。本实验通过蚌埠医科大学动物伦理委员会批准（伦理批号：伦动科批字〔2024〕第297号）。

1.3 小鼠结肠炎症状评分

造模和取检前分别观察并记录小鼠体质量，取检前对小鼠疾病活动指数（DAI）进行评分。DAI评估依据6点评分系统（评分范围为0到5）[17]，以下各项特征各计1分：潜血粪便、毛发蓬乱、软便以及直肠脱垂长度小于1 mm；若直肠脱垂严重且超过1 mm，则额外加1分。

1.4 HE染色

将小鼠结肠组织进行脱水、包埋和切片（厚度为3 μm），经脱蜡、水化，苏木素染色10 min，盐酸乙醇分化3 s，返蓝2 min，伊红30 s，脱水透明后用中性树脂封片。显微镜下观察拍照并进行炎症评分。炎症评分依据0至4分的评分系统评估小鼠肠道炎症程度[18]，其中0分表示无炎症，1分表示固有层中单核细胞浸润极轻微，2分表示因单核细胞浸润导致轻度黏膜增生或隐窝分离，3分表示伴有上皮细胞增生和黏膜结构破坏的中度炎症，4分则涵盖了上述所有特征，并伴有溃疡或脓肿形成。溃疡或隐窝脓肿额外加1分。所有切片均由2名不知情（双盲）的组织病理学家进行评分。

1.5 细胞培养

Caco-2购自BOSTER，在添加20%胎牛血清和1%青霉素-链霉素的MEM培养基中培养。设置培养箱培养条件为5% CO_2，37 ℃。细胞实验分组：Control组（正常培养），LPS组（1 μg/mL LPS干预），10 μmol/L组（1 μg/mL LPS和10 μmol/L NIR干预），25 μmol/L组（1 μg/mL LPS和25 μmol/L NIR干预），50 μmol/L组（1 μg/mL LPS和50 μmol/L NIR干预），LPS+NIR组（1 μg/mL LPS和25 μmol/L NIR干预），Diprovocim组（1 μg/mL LPS、25 μmol/L NIR和110 pmol/L Diprovocim[19]干预），处理后放置细胞培养箱继续培养24 h。

1.6 免疫荧光染色

组织检测：将小鼠结肠组织进行脱水、包埋和切片，经脱蜡、水化，抗原修复后，用5% BSA封闭1 h，一抗［Anti-Bcl-2（1∶400）、Anti-ZO-1（1∶400）；Anti-Claudin-1（1∶400）］孵育过夜，荧光二抗［山羊抗兔/鼠IgG H&L（Alexa Fluor® 555）（1∶1000）］孵育2 h，DAPI染核10 min。抗荧光淬灭剂封片后在荧光显微镜下观察拍照。

细胞检测：细胞接种在爬片上后放置细胞培养箱培养，待细胞汇合至80%终止培养，用4%多聚甲醛固定

20 min，0.2% Triton X-100透膜15 min，封闭1 h，一抗［Anti-Bcl-2（1∶400）、Anti-ZO-1（1∶400）；Anti-Claudin-1（1∶400）］孵育过夜，二抗［山羊抗兔/鼠IgG H&L（Alexa Fluor® 555）（1∶1000）］孵育2 h，DAPI染核10 min，抗荧光淬灭剂封片后在荧光显微镜下观察拍照。

1.7 TUNEL染色

组织检测：取小鼠结肠组织经脱蜡、水化后，根据TUNEL染色试剂盒说明书操作，DAPI染核后用抗荧光淬灭剂封片。于荧光显微镜下采集图像。

细胞检测：从培养箱中取出细胞爬片，先后使用4%多聚甲醛固定和0.3% Triton X-100通透后，按试剂盒说明书进行TUNEL染色。通过荧光显微镜观察并记录图像。

1.8 qRT-PCR

Trizol裂解液提取总RNA，使用PrimeScript™ RT reagent Kit with gDNA Eraser（Perfect Real Time）试剂盒逆转录为cDNA，TB Green™ Premix Ex Taq™ II（TII RNaseH Plus）试剂盒进行扩增，设置参数：95 ℃，30 s，95 ℃，5 s，60 ℃，34 s，40个循环。以GAPDH为内参，使用$2^{-\Delta\Delta Ct}$方法进行统计分析。所有引物由生工生物合成（表1）。

表1 引物序列

Tab.1 Primer sequences for qRT-PCR in this study

Gene	Primer sequences (5'-3')
TNF-α	F: CACGCTCTTCTGTCTACTGAACTTC
	R: CTTGGTGGTTTGTGAGTGTGAGG
IL-1β	F: AATCTCGCAGCAGCACATCAAC
	R: AGGTCCACGGGAAAGACACAG
IL-6	F: GAGAGGAGACTTCACAGAGGATACC
	R: TCATTTCCACGATTTCCCAGAGAAC
IL-10	F: GGACAACATACTGCTAACCGACTC
	R: GGGCATCACTTCTACCAGGTAAAAC
IL-17A	F: TGGCGGCTACAGTGAAGGC
	R: AGGGAGTTAAAGACTTTGAGGTTGAC
GAPDH	F: AACTCCCACTCTTCCACCTTCG
	R: TCCACCACCCTGTTGCTGTAG

1.9 Western blotting

用含蛋白酶抑制剂和磷酸酶抑制剂的RIPA裂解液提取总蛋白，BCA蛋白检测试剂盒测定总蛋白浓度，将蛋白与SDS-上样缓冲液按4∶1混合后高温变性，每组取50 μg总蛋白进行电泳，通过转膜将蛋白转至PVDF膜上，5%脱脂奶粉封闭2 h，一抗［Anti-Bcl-2（1∶2000）、Anti-Bax（1∶2000）、Anti-p38（1∶2000）、Anti-p-p38（1∶2000）Anti-ZO-1（1∶2000）、Anti-Claudin-1（1∶2000）、Anti-p-JNK（1∶2000）、Anti-GAPDH（1∶3000）］孵育过夜，二抗［HRP标记山羊抗兔/鼠IgG（1∶3000）］孵育2 h，使用ECL曝光液在显影仪中显影条带。以GAPDH为内参。Image J进行灰度值分析。

1.10 ELISA炎症因子检测

收集肠黏膜组织，取0.1 g组织加1 mL新鲜配置的RIPA裂解液和蛋白酶抑制剂混合液，充分裂解后离心收集蛋白，根据ELISA试剂盒说明书操作，分别检测IL-6，IL-1β，TNF-α、IL-17A和IL-10的炎症因子水平。

1.11 统计学分析

使用SPSS软件26.0分析数据。数据以均数±标准差表示，组间比较用t检验，多组比较用ANOVA分析，P<0.05表示差异有统计学意义。

2 结果

2.1 NIR干预可改善TNBS诱导小鼠结肠炎症状

与TNBS组相比，TNBS+NIR组小鼠体质量增加，DAI评分减少，结肠长度增加（P<0.05，图1A~D）。较TNBS组小鼠，NIR干预后的TNBS小鼠结肠组织学炎症评分减少（P<0.05，图1E、F）。

2.2 NIR干预可降低TNBS诱导小鼠结肠炎炎症因子水平

qRT-PCR和ELISA检测结果显示，NIR干预后TNBS诱导小鼠结肠黏膜组织中的IL-6，IL-1β、IL-17A和TNF-α的mRNA和蛋白水平降低，并上调了抗炎因子IL-10的表达水平（P<0.05，图2）。

2.3 NIR干预可抑制TNBS诱导小鼠结肠炎肠上皮细胞的凋亡

TUNEL染色检测结果显示，相较于TNBS组，TNBS+NIR组小鼠结肠上皮细胞中凋亡细胞个数较少（P<0.05，图3A、B）。Western blotting和免疫荧光检测结果发现，NIR干预可上调Bcl-2表达，并下调Bax表达（P<0.05，图3C、D）。

2.4 NIR干预可保护TNBS诱导小鼠肠屏障结构

免疫荧光检测结果显示，NIR干预后小鼠结肠黏膜中的ZO-1和Claudin-1分布增多（图4A）。Western blotting检测结果也表明，与TNBS组相比，TNBS+NIR组小鼠结肠中ZO-1和Claudin-1蛋白表达水平升高（P<0.05，图4B）。

2.5 NIR干预可抑制LPS诱导Caco-2细胞的凋亡

TUNEL染色检测结果显示，与LPS组比较，25 μmol/L和50 μmol/L NIR组细胞凋亡数目减少（P<0.05、图5A、B）。Western blotting和免疫荧光检测结果发现，NIR干预可上调LPS诱导Caco-2细胞中Bcl-2表达水平，并下调Bax表达水平（P<0.05，图5C、D）。

2.6 NIR干预可改善LPS诱导Caco-2细胞TJ蛋白的表达

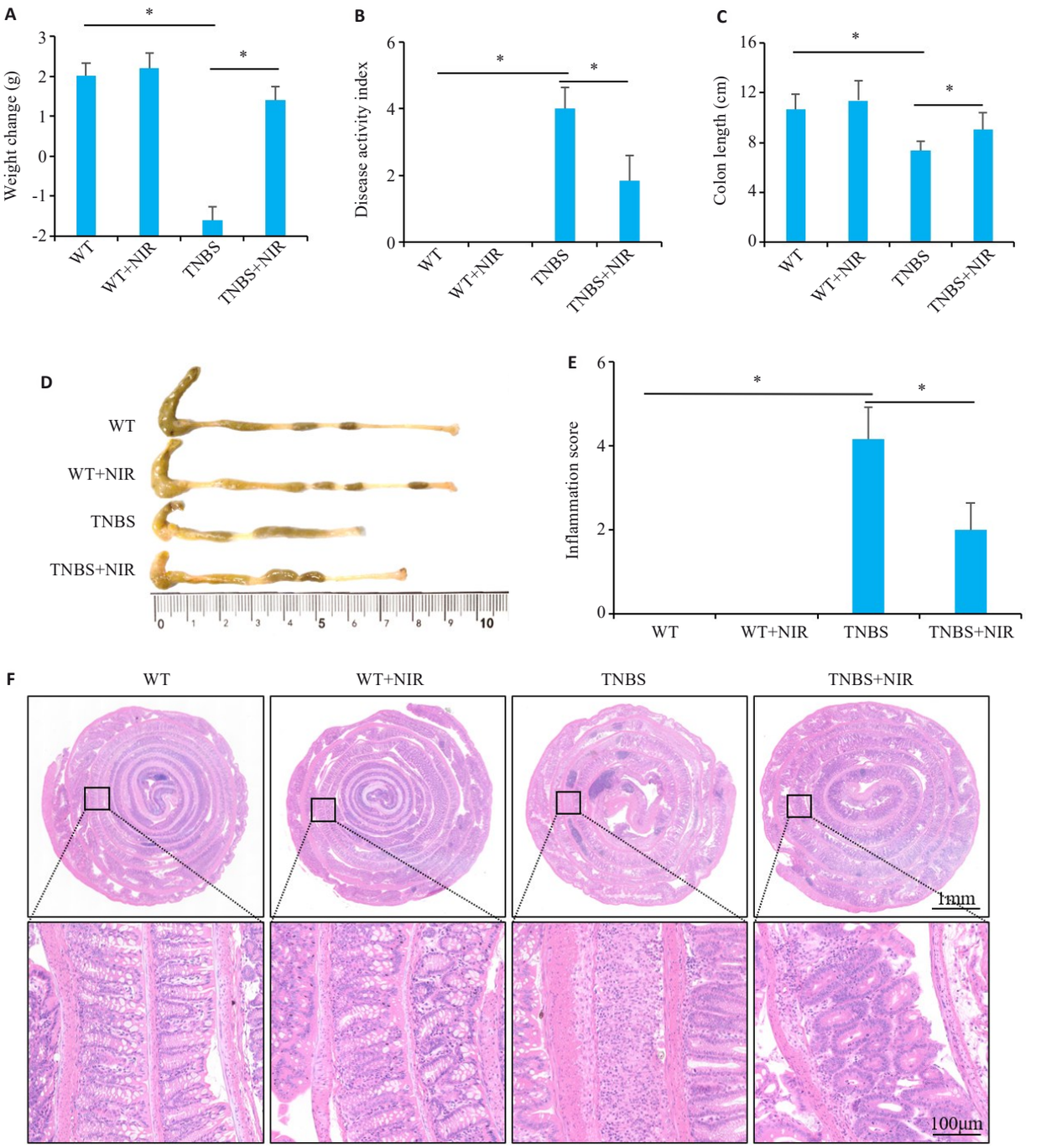

图1　NIR干预对TNBS诱导小鼠结肠炎症状的影响

Fig.1　Effect of niranthin (NIR) treatment on symptoms of TNBS-induced colitis in mice. **A**: Body weight changes of the mice. **B**: DAI score. **C**: Colon length. **D**: Comparison of colon length of the mice among the 4 groups. **E**: Colon inflammation score of the mice. **F**: HE staining of the colon tissues of the mice in the 4 groups. *P<0.05.

细胞免疫荧光和Western blotting检测结果发现，NIR干预可上调LPS诱导Caco-2细胞中ZO-1和Claudin-1表达水平（P<0.05，图6）。

2.7　NIR干预可调控p38/JNK信号

体内研究表明，较TNBS组，TNBS+NIR组p38/JNK蛋白的磷酸化水平降低（P<0.05，图7A）。体外研究进一步证实了，NIR干预可下调LPS诱导Caco-2细胞中p38/JNK蛋白的磷酸化水平（P<0.05，图7B）。Diprovocim干预可缓解NIR对p38/JNK蛋白磷酸化的抑制作用（P<0.05，图7C）。

2.8　NIR干预可通过调控p38/JNK信号改善小鼠CD样肠炎

TUNEL染色和Western blotting检测结果表明，Diprovocim干预后可逆转NIR对肠上皮细胞凋亡的抑制作用（P<0.05，图8A、B）。免疫荧光结果显示，与LPS+NIR组比较，Diprovocim组抗凋亡蛋白Bcl-2以及TJ蛋白ZO-1和Claudin-1表达减弱（图8C、D）。

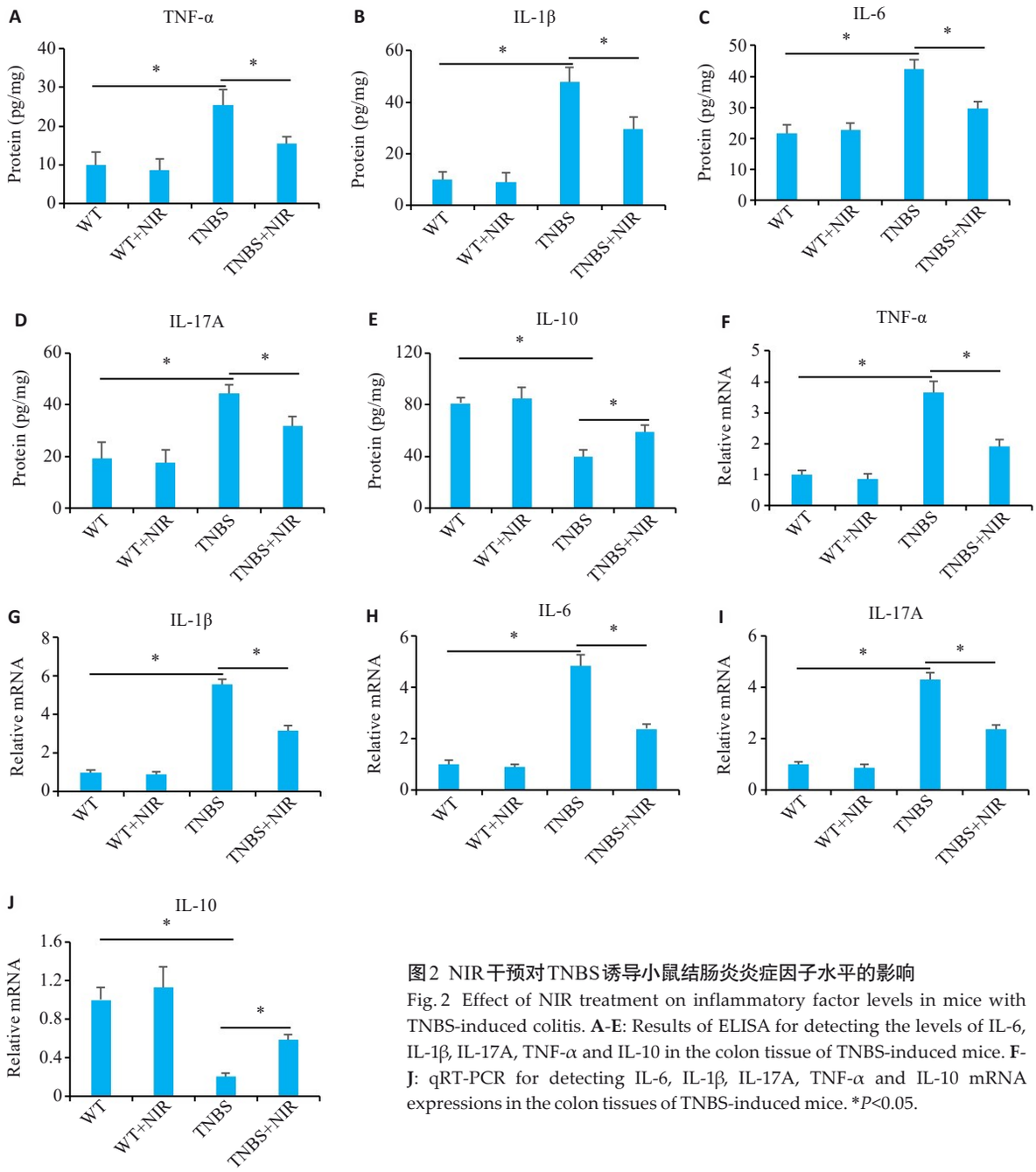

图2 NIR干预对TNBS诱导小鼠结肠炎炎症因子水平的影响

Fig. 2 Effect of NIR treatment on inflammatory factor levels in mice with TNBS-induced colitis. **A**-**E**: Results of ELISA for detecting the levels of IL-6, IL-1β, IL-17A, TNF-α and IL-10 in the colon tissue of TNBS-induced mice. **F**-**J**: qRT-PCR for detecting IL-6, IL-1β, IL-17A, TNF-α and IL-10 mRNA expressions in the colon tissues of TNBS-induced mice. *P<0.05.

3 讨论

本研究表明NIR可改善TNBS诱导小鼠CD样肠炎，减轻肠道炎症反应。研究结果显示，NIR干预可抑制肠上皮细胞凋亡，增强TJ蛋白的表达，从而保护肠屏障。进一步研究发现NIR可通过衰减p38/JNK信号的活化抑制肠上皮细胞凋亡，这可能与NIR改善小鼠CD样肠炎有关。

既往研究报道TNBS诱导小鼠结肠炎模型是一种常用的化学诱导性IBD模型，主要用于CD疾病模型的研究，观察指标包括体质量变化、临床评分、结肠长度、组织病理学和炎症因子等[16, 20]。本研究通过构建TNBS诱导小鼠结肠炎模型，经NIR干预后观察小鼠肠道炎症相关指标，发现TNBS诱导小鼠体质量增加、DAI评分减少、结肠长度增加、炎症评分减少，促炎因子（IL-6，IL-1β、IL-17A和TNF-α）的蛋白和mRNA水平下调并上调抗炎因子IL-10的表达水平。以上阐明NIR可抑制TNBS诱导小鼠的结肠炎症反应。

肠黏膜屏障的调控涉及多个因素，其中以机械屏障最为重要，主要由肠上皮细胞、细胞间紧密连接和黏液层构成[21-23]。值得关注的是，肠上皮细胞形成单层柱状上皮，并通过紧密连接封闭细胞间隙，阻止肠腔内有害物质如细菌和毒素的侵入，这一机制在CD发病过程中具有关键调控作用[22, 24]。在本研究中，基于TNBS诱导小鼠结肠炎模型和LPS诱导Caco-2细胞模型，我们发现NIR干预可抑制肠上皮细胞的凋亡，Western blotting检测进一步揭示NIR可上调Bcl-2表达，并下调Bax表

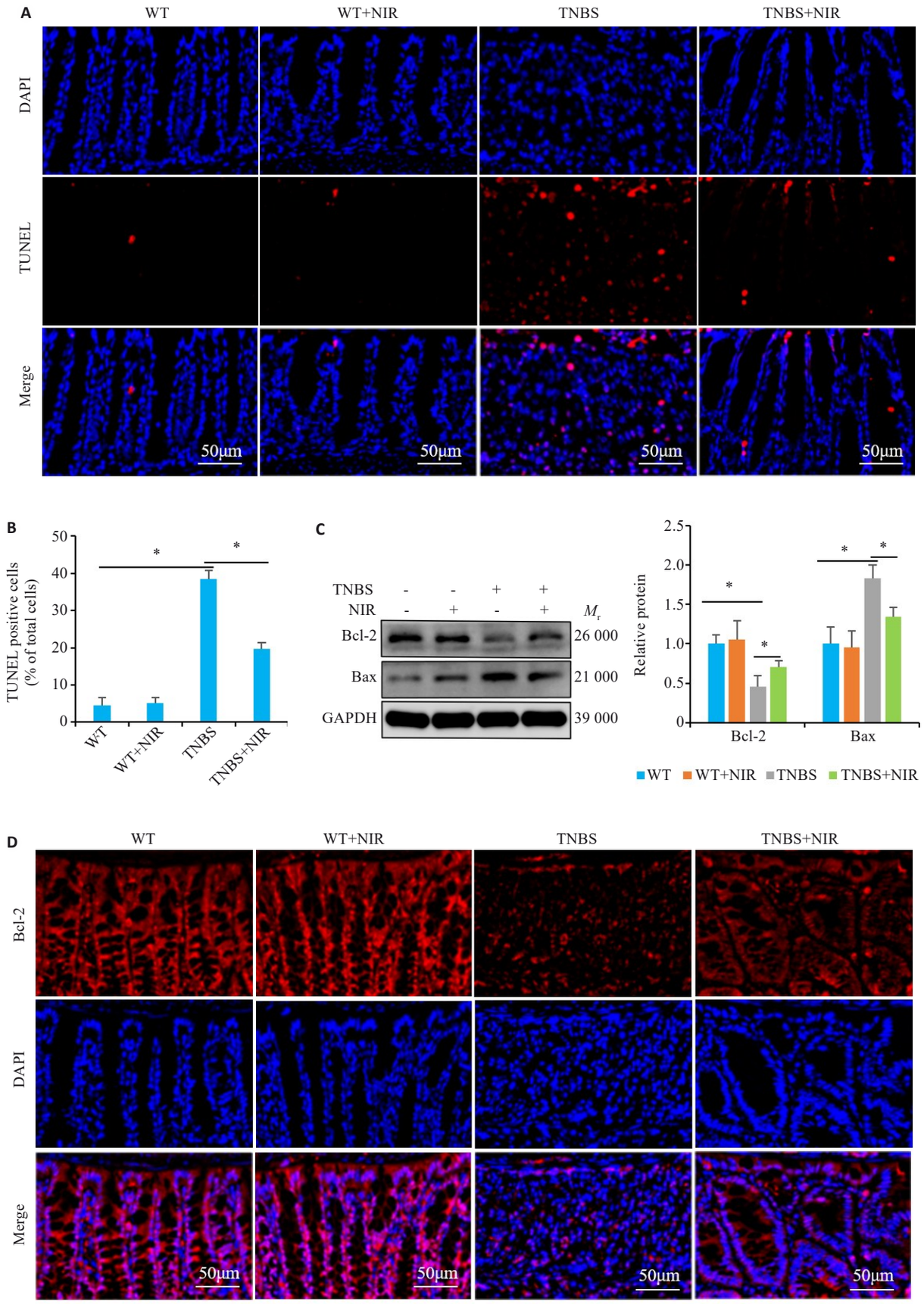

图3 NIR干预对TNBS诱导小鼠结肠炎肠上皮细胞凋亡的影响

Fig.3 Effect of NIR treatment on TNBS-induced apoptosis of intestinal epithelial cells in the mouse models of colitis. **A**, **B**: TUNEL staining for detecting apoptosis in TNBS-induced mice with NIR treatment. **C**: Western blotting for detecting the expression of Bcl-2 and Bax proteins in TNBS-induced mice with NIR treatment. **D**: Immunofluorescence staining for detecting Bcl-2 expression in TNBS-induced mice with NIR treatment. *P<0.05.

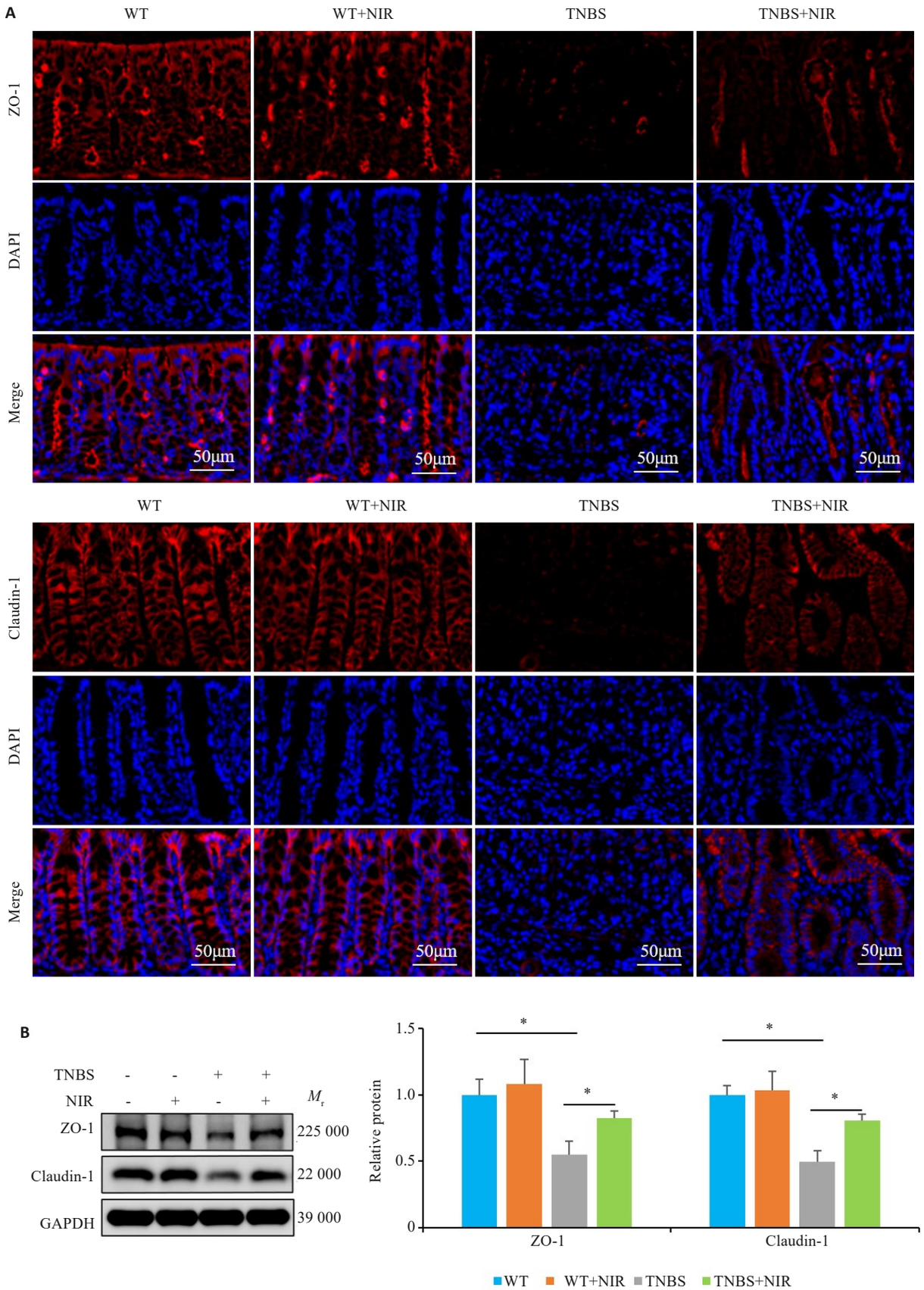

图4 NIR干预对TNBS诱导小鼠结肠炎肠屏障的影响

Fig.4 Effect of NIR treatment on intestinal barrier function in mice with TNBS-induced colitis. **A**: Immunofluorescence staining for detecting ZO-1 and Claudin-1 expression in TNBS-induced mice with NIR treatment. **B**: Western blotting for detecting ZO-1 and claudin-1 protein expressions in TNBS-induced mice with NIR treatment. *P<0.05.

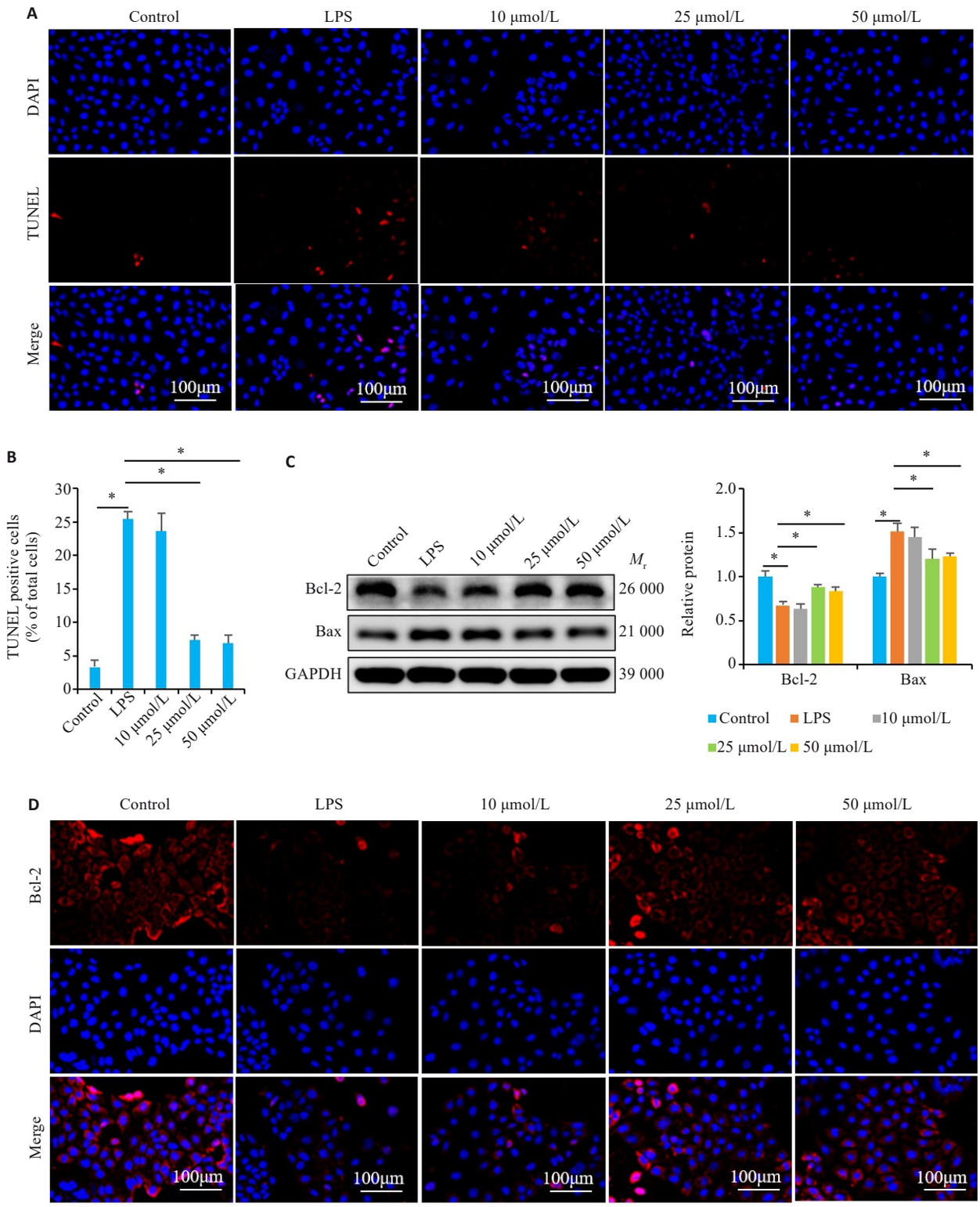

图5　NIR干预对LPS诱导Caco-2细胞凋亡的影响

Fig. 5　Effect of NIR treatment on LPS-induced apoptosis in Caco-2 cells. **A**, **B**: TUNEL staining for detecting LPS-induced apoptosis of Caco-2 cells with NIR treatment. **C**: Western blotting for detecting the expression of Bcl-2 and Bax proteins in LPS-induced Caco-2 cells with NIR treatment. **D**: Immunofluorescence staining for detecting Bcl-2 expression in LPS-induced Caco-2 cells with NIR treatment. *P<0.05.

达。这些结果证实NIR对CD样肠炎具有抗凋亡作用。值得注意的是，既往研究表明，肠上皮细胞凋亡过度会导致肠屏障破坏，进而使得肠道通透性增加[25, 26]。与本发现相呼应，Zeng等[27]证实了PHLDA1通过限制炎症性肠病中肠上皮细胞凋亡来保护肠道屏障功能。GK可通过减少凋亡细胞数量以及抑制关键凋亡调控因子的表达来抑制肠上皮细胞凋亡，改善肠通透性并保护肠道屏障[28]。更有趣的是，本研究发现NIR不仅能调控凋亡

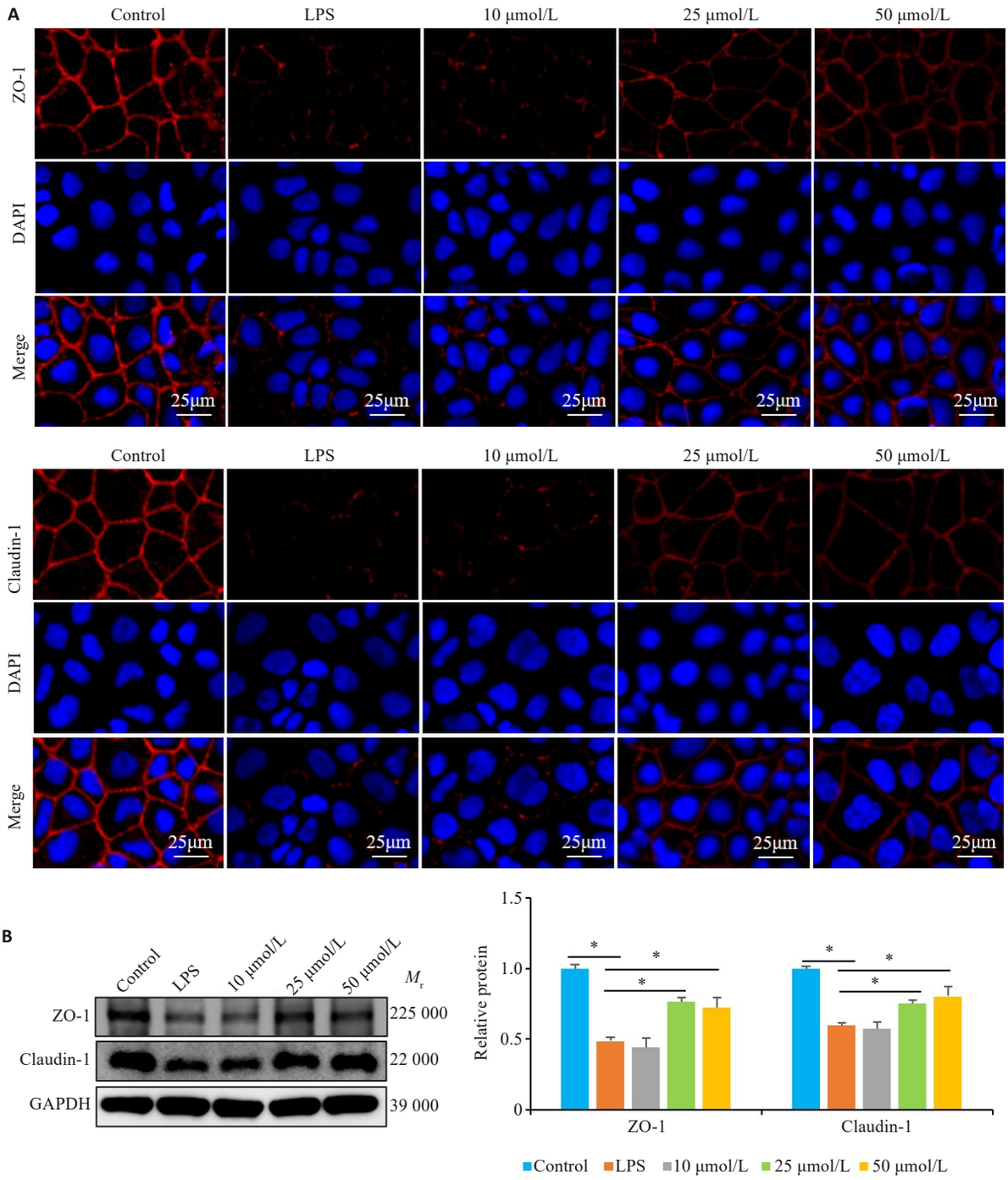

图6 NIR干预对LPS诱导Caco-2细胞TJ蛋白表达的影响

Fig. 6 Effect of NIR treatment on expressions of tight junction proteins in LPS-induced Caco-2 cells. **A**: Immunofluorescence staining showing ZO-1 and claudin-1 expressions in LPS-induced Caco-2 cells with NIR treatment. **B**: Western blotting for detecting ZO-1 and claudin-1 protein expressions in LPS-induced Caco-2 cells with NIR treatment. *P<0.05.

相关蛋白表达，还通过免疫荧光和Western blotting检测分析发现NIR可增强TJ蛋白（ZO-1和Claudin-1）的表达。这些结果进一步阐明NIR可通过抑制肠上皮细胞凋亡发挥保护肠屏障的作用。

既往研究报道，MAPK（丝裂原活化蛋白激酶）通路在肠道炎症和细胞凋亡中扮演着关键角色[29, 30]。研究表明，JNK和p38 MAPK通路的激活可以通过多种途径诱导细胞凋亡，参与细胞生存和炎症反应等[31, 32]。Tao等[33]证实了百菌清（CTL）通过激活MAPK信号通路诱导肠上皮细胞凋亡，包括下调抗凋亡基因（BCL-2）的mRNA水平，上调与凋亡相关的基因（包括BAD、BAX、CASP3和CASP8）的mRNA水平。Chu等[32]发现GA通过抑制LPS诱导的Caco-2细胞中MAPK信号通路中关键信号分子（包括p38、JNK和ERK）的磷酸化，减少细胞的早期和晚期凋亡比例、下调促凋亡因子（Bax、Bad、caspase-3、caspase-8和caspase-9）的mRNA

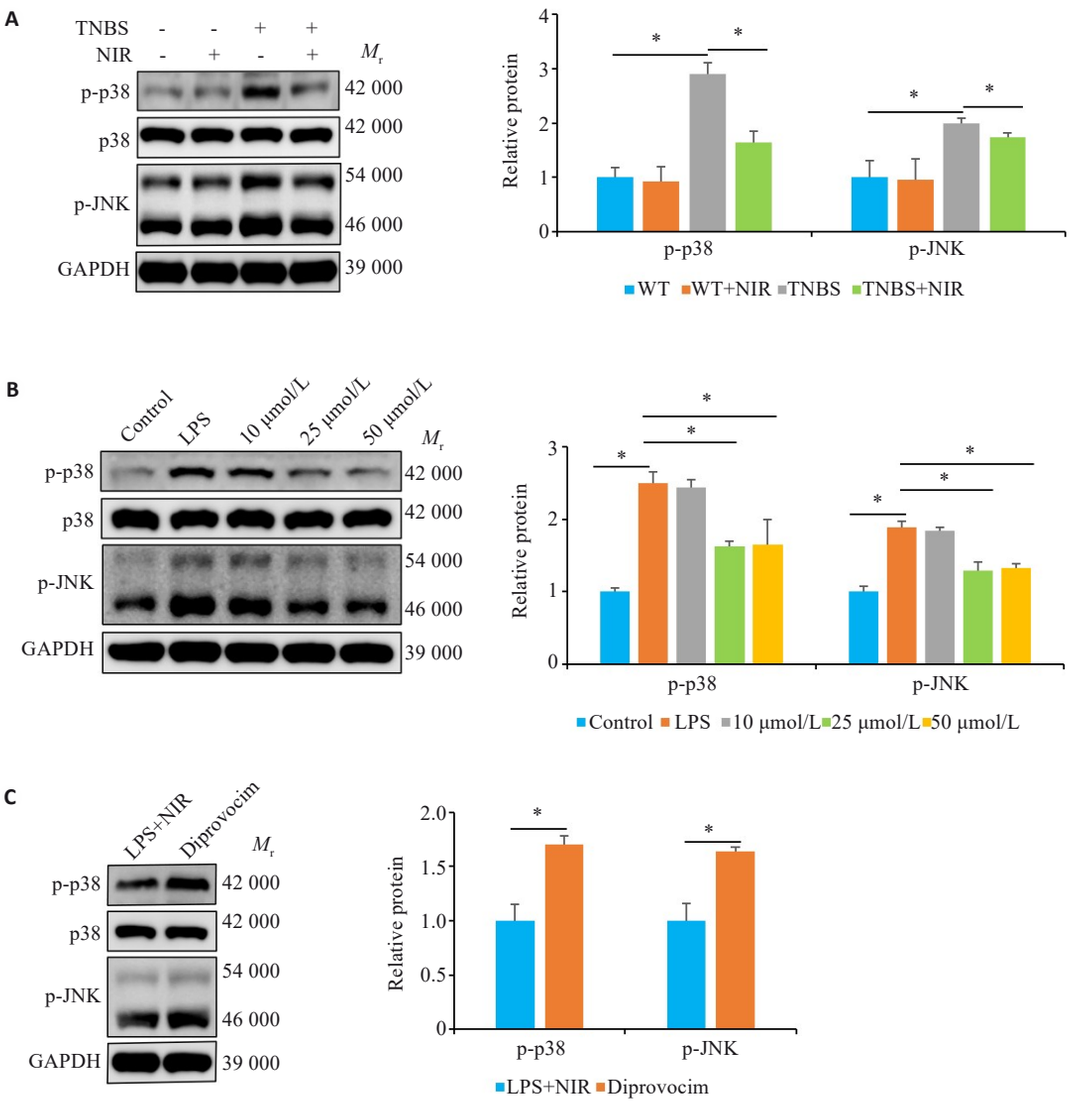

图7　NIR干预可调控p38/JNK信号

Fig. 7　NIR treatment modulates p38/JNK signaling. **A**: Western blotting for detecting p-p38 and p-JNK protein expressions in TNBS-induced mice with NIR treatment. **B**: Western blotting for detecting p-p38 and p-JNK protein expressions in LPS-induced Caco-2 cells with NIR treatment. **C**: Western blotting for detecting p-p38 and p-JNK protein expressions in the colon tissues of the mice with NIR and diprovocim treatment. *P<0.05.

水平以及上调抗凋亡因子Bcl-2的mRNA水平，发挥其抗炎作用。基于上述机制洞悉，本研究深入探讨NIR抑制肠道炎症的潜在分子机制，Western blotting检测分析发现，NIR干预后可抑制p38/JNK蛋白磷酸化，Diprovocim干预可衰减NIR对p38/JNK通路的失活作用；同时，TUNEL染色和Western blotting检测分析发现，Diprovocim干预后可逆转NIR对Caco-2细胞凋亡的抑制作用，逆转了NIR对Bcl-2蛋白表达的促进作用，以及对Bax蛋白表达的抑制作用。以上发现证实NIR可通过抑制p38/JNK信号活化促进抗凋亡通路的激活。

本研究通过体内外实验证实了NIR可抑制肠上皮细胞凋亡，并可能与p38/JNK信号通路失活有关，为揭示IBD的发病机制提供新思路；NIR可促进肠屏障的修复，从而改善肠道炎症反应，为临床上CD患者的药物治疗提供新的理论依据。但本研究也存在一些局限性，包括本研究只探讨了NIR对肠上皮细胞凋亡的作用，尚不能排除通过其他功能学途径发挥作用；本研究仅证实了NIR抑制p38/JNK信号活化发挥作用，可能忽视了NIR调控其他分子机制发挥作用。NIR作为一种具有多重生物活性的天然化合物，其临床转化之路虽面临生物利用度、安全性和药效稳定性等挑战，但未来研究通过多学科协作，整合药物化学、药剂学、分子药理学和临床医学等多领域专业知识可逐步攻克，加速NIR的临床转化进程。

综上所述，NIR可抑制肠上皮细胞凋亡，改善肠道炎症，其潜在的分子机制可能与p38/JNK信号通路有关。

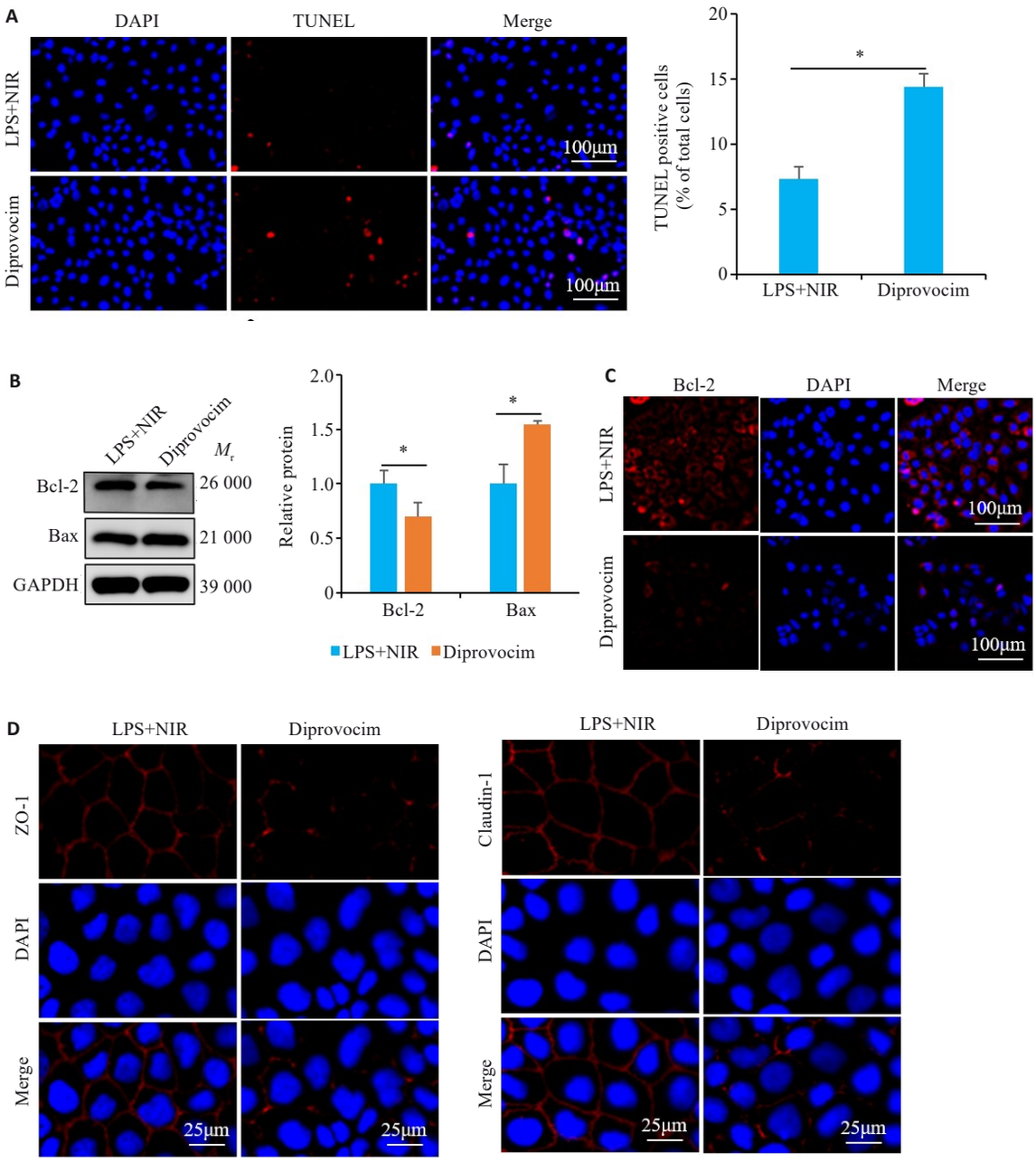

图8 Diprovocim干预对NIR治疗Caco-2细胞中凋亡和TJ蛋白表达的影响

Fig. 8 Effects of diprovocim intervention on apoptosis and expressions of tight junction proteins in NIR-treated Caco-2 cells. **A**: TUNEL staining for detecting apoptosis in NIR-treated Caco-2 cells by Diprovocim treatment. **B**: Western blotting for detecting the expression of Bcl-2 and Bax proteins in NIR-treated Caco-2 cells with diprovocim treatment. **C**, **D**: Immunofluorescence staining for detecting Bcl-2, ZO-1 and Claudin-1 expression in NIR-treated Caco-2 cells with diprovocim treatment. **P*<0.05.

Declaration of interests: The authors declare no competing interests.

参考文献：

[1] Dolinger M, Torres J, Vermeire S. Crohn's disease[J]. Lancet, 2024, 403(10432): 1177-91.

[2] Loftus EV Jr, Panés J, Lacerda AP, et al. Upadacitinib induction and maintenance therapy for Crohn's disease[J]. N Engl J Med, 2023, 388(21): 1966-80.

[3] Honap S, Jairath V, Danese S, et al. Navigating the complexities of drug development for inflammatory bowel disease[J]. Nat Rev Drug Discov, 2024, 23(7): 546-62.

[4] Wang L, Song X, Zhou YQ, et al. Sclareol protected against intestinal barrier dysfunction ameliorating Crohn's disease-like colitis *via* Nrf2/NF-B/MLCK signalling[J]. Int Immunopharmacol, 2024, 133: 112140.

[5] Zhou L, Zhu L, Wu X, et al. Decreased TMIGD1 aggravates colitis and intestinal barrier dysfunction *via* the BANF1-NF-κB pathway in Crohn's disease[J]. BMC Med, 2023, 21(1): 287.

[6] Xu S, Peng YF, Yang K, et al. PROTAC based STING degrader attenuates acute colitis by inhibiting macrophage M1 polarization and intestinal epithelial cells pyroptosis mediated by STING-NLRP3 axis[J]. Int Immunopharmacol, 2024, 141: 112990.

[7] Tan G, Huang C, Chen J, et al. HMGB1 released from GSDME-mediated pyroptotic epithelial cells participates in the tumorigenesis of colitis-associated colorectal cancer through the ERK1/2 pathway [J]. J Hematol Oncol, 2020, 13(1): 149.

[8] Liu J, Di B, Xu LL. Recent advances in the treatment of IBD: targets, mechanisms and related therapies[J]. Cytokine Growth Factor Rev, 2023, 71: 1-12.

[9] Veyrard P, Nancey S, Roblin X. Editorial: 5-ASA in IBD patients on biologics-' stop or continue'[J]? Aliment Pharmacol Ther, 2021, 54 (6): 843-4.

[10] Sridhar A, Bakke I, Gopalakrishnan S, et al. Tofacitinib and budesonide treatment affect stemness and chemokine release in IBD patient-derived colonoids[J]. Sci Rep, 2025, 15(1): 3753.

[11] Biologic therapy for inflammatory bowel disease: real-world comparative effectiveness and impact of drug sequencing in 13 222 patients within the UK IBD BioResource[J]. J Crohns Colitis, 2024, 18(6): 790-800.

[12] Ota R, Karasawa D, Oshima M, et al. Asymmetric total synthesis of four bioactive lignans using donor–acceptor cyclopropanes and bioassay of (−)- and (+)-niranthin against hepatitis B and influenza viruses[J]. RSC Adv, 2022, 12(8): 4635-9.

[13] Harikrishnan H, Jantan I, Alagan A, et al. Modulation of cell signaling pathways by *Phyllanthus* amarus and its major constituents: potential role in the prevention and treatment of inflammation and cancer[J]. Inflammopharmacology, 2020, 28(1): 1-18.

[14] Chowdhury S, Mukherjee T, Mukhopadhyay R, et al. The lignan niranthin poisons *Leishmania donovani* topoisomerase IB and favours a Th1 immune response in mice[J]. EMBO Mol Med, 2012, 4(10): 1126-43.

[15] Harikrishnan H, Jantan I, Haque MA, et al. Anti-inflammatory effects of hypophyllanthin and niranthin through downregulation of NF- κB/MAPKs/PI3K-Akt signaling pathways[J]. Inflammation, 2018, 41(3): 984-95.

[16] Tan G, Huang CY, Chen JY, et al. Gasdermin-E-mediated pyroptosis participates in the pathogenesis of Crohn's disease by promoting intestinal inflammation[J]. Cell Rep, 2021, 35(11): 109265.

[17] Spencer DM, Veldman GM, Banerjee S, et al. Distinct inflammatory mechanisms mediate early versus late colitis in mice[J]. Gastroenterology, 2002, 122(1): 94-105.

[18] Rath HC, Herfarth HH, Ikeda JS, et al. Normal luminal bacteria, especially *Bacteroides* species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats[J]. J Clin Invest, 1996, 98(4): 945-53.

[19] Morin MD, Wang Y, Jones BT, et al. Diprovocims: A New and Exceptionally Potent Class of Toll-like Receptor Agonists[J]. J Am Chem Soc, 2018, 140(43):14440-54.

[20] Wei ZY, Ni X, Cui H, et al. Engeletin attenuates the inflammatory response *via* inhibiting TLR4-NFκB signaling pathway in Crohn's disease-like colitis[J]. J Ethnopharmacol, 2025, 336: 118733.

[21] Mehandru S, Colombel JF. The intestinal barrier, an arbitrator turned provocateur in IBD[J]. Nat Rev Gastroenterol Hepatol, 2021, 18(2): 83-4.

[22] Odenwald MA, Turner JR. The intestinal epithelial barrier: a therapeutic target[J]? Nat Rev Gastroenterol Hepatol, 2017, 14(1): 9-21.

[23] Huang S, Xie Z, Han J, et al. Protocadherin 20 maintains intestinal barrier function to protect against Crohn's disease by targeting ATF6 [J]. Genome Biol, 2023, 24(1): 159.

[24] Safari F, Sharifi M, Talebi A, et al. Alleviation of cholestatic liver injury and intestinal permeability by lubiprostone treatment in bile duct ligated rats: role of intestinal FXR and tight junction proteins claudin-1, claudin-2, and occludin[J]. Naunyn Schmiedeberg's Arch Pharmacol, 2023, 396(9): 2009-22.

[25] Pan Z, Huang J, Hu T, et al. Protective effects of selenium nanoparticles against bisphenol A-induced toxicity in porcine intestinal epithelial cells[J]. Int J Mol Sci, 2023, 24(8): 7242.

[26] Yin M, Shen Z, Yang L, et al. Protective effects of *CXCR3*/HO-1 gene-modified BMMSCs on damaged intestinal epithelial cells: Role of the p38-MAPK signaling pathway[J]. Int J Mol Med, 2019, 43(5): 2086-102.

[27] Zeng ZW, Shi YM, Cai YH, et al. PHLDA1 protects intestinal barrier function *via* restricting intestinal epithelial cells apoptosis in inflammatory bowel disease[J]. Exp Cell Res, 2024, 443(1): 114322.

[28] Geng Z, Zuo L, Li J, et al. Ginkgetin improved experimental colitis by inhibiting intestinal epithelial cell apoptosis through EGFR/PI3K/AKT signaling[J]. FASEB J, 2024, 38(14): e23817.

[29] Xu Q, Liu M, Chao X, et al. Stevioside improves antioxidant capacity and intestinal barrier function while attenuating inflammation and apoptosis by regulating the NF- κB/MAPK pathways in diquat-induced oxidative stress of IPEC-J2 cells[J]. Antioxidants: Basel, 2023, 12(5): 1070.

[30] Xing Z, Li X, He J, et al. OLFM4 modulates intestinal inflammation by promoting IL-22^{+}ILC3 in the gut[J]. Commun Biol, 2024, 7 (1): 914.

[31] Xiao L, Zhang WH, Huang Y, et al. Intestinal ischemia-reperfusion induces the release of IL-17A to regulate cell inflammation, apoptosis and barrier damage[J]. Exp Ther Med, 2022, 23(2): 158.

[32] Chu C, Ru H, Chen Y, et al. Gallic acid attenuates LPS-induced inflammation in Caco-2 cells by suppressing the activation of the NF-κB/MAPK signaling pathway[J]. Acta Biochim Biophys Sin: Shanghai, 2024, 56(6): 905-15.

[33] Tao H, Bao Z, Fu Z, et al. Chlorothalonil induces the intestinal epithelial barrier dysfunction in Caco-2 cell-based *in vitro* monolayer model by activating MAPK pathway[J]. Acta Biochim Biophys Sin: Shanghai, 2021, 53(11): 1459-68.

（编辑:余诗诗）