[文章编号] 1671-587X(2025)01-0076-09 DOI:10.13481/j.1671-587X.20250110

铁皮石斛多糖通过调节肠菌代谢物对老年小鼠肠道黏膜屏障损伤的改善作用

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[摘 要] **目的：**探讨铁皮石斛多糖（DOP）对老年小鼠肠道黏膜屏障损伤的保护作用，并阐明其可能的作用机制。**方法：**选择10只雌性5月龄C57BL/6小鼠作为年轻组，将20只雌性C57BL/6小鼠（15月龄）随机分为老年组和老年鼠DOP（200 mg·kg^{-1}·d^{-1}）处理组（DOP组），每组10只。DOP组小鼠采用DOP灌胃，检测各组小鼠体质量、摄食量和悬挂时间；HE染色观察各组小鼠肠道和脾脏组织病理形态表现；免疫组织化学染色检测各组小鼠肠道组织中闭锁小带蛋白1（ZO-1）和黏蛋白2（MUC2）的表达情况。制备肠菌代谢物培养基（IBMM）干预秀丽线虫（*C. elegans*），将*C. elegans*随机分为年轻IBMM组（Young-IBMM）、老年IBMM组（Aged-IBMM）和DOP-IBMM组，采用免疫荧光法分析各组*C. elegans*第1和12天的肠道脂褐素累积水平；亮蓝染色检测各组*C. elegans*第1和12天的肠道渗漏情况。制备IBMM干预Caco-2细胞，将Caco-2细胞分为Young-IBMM组、Aged-IBMM组和DOP-IBMM组，采用Western blotting法检测各组Caco-2细胞中ZO-1、闭合蛋白（Occludin）、肿瘤坏死因子α（TNF-α）、白细胞介素6（IL-6）、磷酸化肌球蛋白轻链（p-MLC）和肌球蛋白轻链激酶（MLCK）蛋白表达水平。**结果：**与年轻组比较，老年组小鼠体质量增加（$P<0.05$），摄食量降低（$P<0.05$），悬挂时间缩短（$P<0.05$）；与老年组比较，DOP组小鼠体质量明显减轻（$P<0.01$），摄食量增加（$P<0.05$），悬挂时间明显延长（$P<0.01$）。HE染色，与年轻组比较，老年组小鼠肠黏膜厚度变薄，杯状细胞减少，肠绒毛长短不一且排列无序，脾脏表面可见大量铁血黄素存在，红髓内细胞成分减少，白髓内动脉周围淋巴鞘和淋巴小结残存或几乎消失；与老年组比较，DOP组小鼠肠黏膜厚度增加，杯状细胞增多，肠绒毛长度一致且排列整齐，脾脏红髓整体功能改善，白髓成分增加。免疫组织化学染色，与年轻组比较，老年组小鼠肠道组织中ZO-1和MUC2蛋白表达水平明显降低（$P<0.05$或$P<0.001$）；与老年组比较，DOP组小鼠肠道组织中ZO-1和MUC2蛋白表达水平明显升高（$P<0.05$或$P<0.001$）。免疫荧光法，与Young-IBMM组比较，Aged-IBMM组*C. elegans*肠道内脂褐素累积水平明显升高（$P<0.001$）；与Aged-IBMM组比较，DOP-IBMM组*C. elegans*肠道内脂褐素累积水平明显降低（$P<0.001$）。亮蓝染色法，与Young-IBMM组比较，Aged-IBMM组*C. elegans*亮蓝染料渗漏至线虫全身，肠道结构模糊不清，不易观察；与Aged-IBMM组比较，DOP-IBMM组*C. elegans*亮蓝染料渗漏减少。Western blotting法，与Young-IBMM组比较，Aged-IBMM组Caco-2细胞中TNF-α、IL-6、p-MLC和MLCK蛋白表达水平明显升高（$P<0.01$或$P<0.001$），ZO-1和Occludin蛋白表达水平明显降低（$P<0.05$或$P<0.01$）；与Aged-IBMM组比较，DOP-IBMM组

[收稿日期] 2024-01-19 [录用日期] 2024-04-14
[基金项目] 吉林省教育厅科学技术研究项目（JJKH20230984KJ）；吉林省科技厅科技发展计划项目（YDZJ202301ZYTS446）
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Caco-2细胞中TNF-α、IL-6、p-MLC和MLCK蛋白表达水平明显降低（$P<0.01$），ZO-1和Occludin蛋白表达水平明显升高（$P<0.05$或$P<0.01$）。**结论：**DOP对老年小鼠肠道黏膜屏障损伤有改善作用，其作用机制可能与通过调节肠菌代谢物、抑制p-MLC/MLCK通路、恢复紧密连接复合物表达、降低肠道炎症水平、进而改善肠道屏障损伤有关。

[关键词]　铁皮石斛多糖；自然衰老小鼠；肠道屏障；肠菌代谢物

[中图分类号]　R965　　**[文献标志码]**　A

Ameliorating effect of *Dendrobium officinale* polysaccharides on intestinal mucosal barrier damage in elderly mice by regulating intestinal microbial metabolites

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ABSTRACT　**Objective:** To investigate the protective effect of *Dendrobium officinale* polysaccharides (DOP) on intestinal mucosal barrier damage, and to elucidate the possible mechanism. **Methods:** Ten female C57BL/6 mice, aged 5 months, were selected as young group; twenty femal C57BL/6 mice, aged 15 months, were randomly divided into aged group and DOP treatment group (200 $mg \cdot kg^{-1}$, DOP group), with 10 mice in each group. The mice in DOP group were administrated with DOP by gavage. The body mass, food intakes and hanging time of the mice in various groups were detected. HE staining was used to observe the pathomorphology of intestinal and spleen tissues of the mice in various groups. Immunohistochemical staining was used to detect the expressions of intestinal atresin 1 (ZO-1) and Mucin 2 (MUC2) in intestinal tissue of the mice in various groups. The intestinal baterial metabolite medium (IBMM) were prepared to intervene the *Caenorhabditis elegans* (*C. elegans*), and the *C. elegans* were randomly divided into Young-IBMM group, Aged-IBMM group, and DOP-IBMM group. Immunofluorescence method was used to analyze the intestinal lipofuscin accumulation levels on the 1st day and the 12th day of the *C. elegans* in various groups. Brilliant blue staining was used to assess the intestinal leakage on the 1st day and the 12th day of *C. elegans* in various groups. The Caco-2 cells were randomly divided into Young-IBMM, Aged-IBMM and DOP-IBMM groups, and Western blotting method was used to detect the expression levels of ZO-1, Occludin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), phosphorylated myosin light chain (p-MLC), myosin light chain kinase (MLCK) proteins in the Caco-2 cells in various groups. **Results:** Compared with young group, the body mass of the mice in aged group was increased ($P<0.05$), the amount of food intake was decreased ($P<0.05$), and the hanging time was decreased ($P<0.05$); compared with aged group, the body mass of the mice in DOP group was significantly decreased ($P<0.01$), the amount of food intake was increased ($P<0.05$), and the hanging time was significantly extended ($P<0.01$). The HE staining results showed that compared with young group, the thickness of intestinal mucosa of the mice in aged group became thinner, the goblet cells were reduced, the intestinal villi were disordered with different lengths, a large amount of hemosiderin was found on the surface of the spleen, the cell components in the red medullary were reduced, and the lymphatic sheath and lymphatic nodes around the intra-white pulp artery remained or almost disappeared; compared with aged group, the thickness of the intestinal mucosa of the mice in DOP group was increased, the goblet

cells were increased, the length of the intestinal villi was consistent and neatly arranged, the overall function of the red pulp of the spleen was improved, and the components of the white pulp were increased. The immunohistochemical staining results showed that compared with young group, the expression levels of ZO-1 and MUC2 proteins in intestinal tissue of the mice in aged group were significantly decreased ($P<0.05$ or $P<0.001$); compared with aged group, the expression levels of ZO-1 and MUC2 proteins in the intestinal tissue of the mice in DOP group were significantly increased ($P<0.05$ or $P<0.001$). The immuno-fluorescence analysis showed that compared with Young-IBMM group, the intestinal lipofuscin accumulation level of *C. elegans* in Aged-IBMM group was significantly increased ($P<0.001$); compared with Aged-IBMM group, the intestinal lipofuscin accumulation level of *C. elegans* in DOP-IBMM group was significantly reduced ($P<0.001$). The brilliant blue staining showed that compared with Young-IBMM group, the bright blue dye leaked into the whole body of *C. elegans* from intestinal tissue in Aged-IBMM group, and the intestinal structure became blurred and was difficulted to be observed; compared with Aged-IBMM group, the leakage of bright blue dye of *C. elegans* in DOP-IBMM was reduced. The Western blotting results showed that compared with Young-IBMM group, the expression levels of TNF-α, IL-6, p-MLC, and MLCK proteins in the Caco-2 cells in Aged-IBMM group were significantly increased ($P<0.01$ or $P<0.001$), and the expression levels of ZO-1 and Occludin proteins were significantly decreased ($P<0.05$ or $P<0.01$); compared with Aged-IBMM, the expression levels of TNF-α, IL-6, p-MLC and MLCK proteins in the Caco-2 cells in DOP-IBMM group were significantly decreased ($P<0.01$), and the expression levels of ZO-1 and Occludin proteins were significantly increased ($P<0.05$ or $P<0.01$). **Conclusion**: DOP has an ameliorating effect on intestinal mucosal barrier damage in the aged mice, and its mechanism may be related to the improvement of intestinal barrier damage by regulating intestinal bacterial metabolites, inhibiting the p-MLC/MLCK signal pathway, restoring the expression of tight junction complexes, and reducing the level of intestinal inflammation.

KEYWORDS *Dendrobium officinale* polysaccharides; Natural aging mice; Intestinal barrier; Intestinal bacterial metabolites

衰老伴随着生理功能下降和多种慢性疾病发病率的激增[1]。肠道稳态是衰老进程的决定性因素之一。肠道黏膜屏障损伤与肠道微生态失衡相互促进并加剧了全身慢性炎症。因此，保护肠道屏障完整性对于延缓衰老尤为重要[2]。铁皮石斛为兰科石斛属植物的干燥茎，是中国传统名贵中药之一，具有抗炎、促进氧化平衡和改善糖脂代谢等作用[3]，现已成为研究热点。铁皮石斛多糖（*Dendrobium officinale* polysaccharides，DOP）是铁皮石斛的主要活性成分[4]，可通过调控肠道菌群结构增加有益代谢产物丰度，进而改善病原体渗漏及代谢性炎症水平，对肠道炎症性疾病具有一定的治疗作用[5]。LIANG等[6]发现：DOP可通过抑制NOD样受体热蛋白结构域相关蛋白3（NOD-like receptor thermal protein domain associated protein 3，NLRP3）炎性体和β抑制蛋白1（β-arrestin 1，ARRB1）信号通路，显著改善葡聚糖硫酸钠（dextran sulfate sodium，DSS）诱导的急性结肠炎（ulcerative colitis，UC）小鼠结肠病理损伤，降低其死亡率。但有关DOP在衰老肠道屏障损伤的调控作用目前报道较少。本研究选取DOP对老年小鼠进行干预，观察其对老年小鼠肠道屏障结构损伤的改善作用，并通过制备肠菌代谢物培养基（intestinal bacterial metabolite medium，IBMM）利用秀丽线虫（*Caenorhabditis elegans*，*C. elegans*）及人结直肠腺癌（Caco-2）细胞模型探讨其对肠道屏障损伤保护的作用机制，为延缓衰老并提高健康生命周期质量提供参考。

1 材料与方法

1.1 实验动物、主要试剂和仪器 30只SPF级雌性C57BL/6小鼠，购自北京维通利华实验动物技术有限公司，动物生产许可证号：SCXK（京）2021-0006，北京市实验动物质量合格证：110011231109707186，小鼠饲养于吉林省长春市农业科学研究院，动物实验获得长春中医药大学动物伦理委员会批准，批准号：JLSZKYDWLL2023-049。石油醚、三氯甲烷、正丁醇和氯化钠（北京国药集

团股份有限公司），乙醇（济南世纪通达化工有限公司），磷酸盐缓冲液（phosphate buffer saline，PBS）（上海达特希尔生物科技有限公司），二氨基联苯胺（diaminobenzidine，DAB）（北京普利莱基因技术有限公司），木瓜蛋白酶和葡萄糖（美国Sigma公司），无水乙醇（上海安谱实验科技股份有限公司），闭锁小带蛋白1（zonula occluden-1，ZO-1）、黏蛋白2（mucin 2，MUC2）、闭合蛋白（Occludin）、肿瘤坏死因子α（tumor necrosis factor-α，TNF-α）、白细胞介素6（interleukin-6，IL-6）、肌球蛋白轻链（myosin light chain，MLC）、磷酸化MLC（phosphorylated MLC，p-MLC）、MLC激酶（MLC kinase，MLCK）和GAPDH抗体均购自美国Proteintech公司。电子天平（型号：ESJ30-5A，沈阳神宇龙腾天平有限公司），酶标仪（型号：Multiskan GO，美国Thermo公司），纯水仪（型号：Milli-Q，美国Merck公司），冷冻干燥机（型号：DGJ-10C，上海博登生物科技有限公司），旋转蒸发仪（型号：RE 5298A，上海亚荣生化仪器有限公司），AKTA纯化系统（型号：explorer 100，美国GE公司）。

1.2　DOP的制备　将烘干后铁皮石斛茎用粉碎机粉碎，并过60目筛。加入无水乙醇，搅拌萃取脂溶性色素和部分杂质，离心收集沉淀。向沉淀中加入纯水，60 ℃水浴提取4 h，离心收集上清提取液。沉淀残渣按照同样的步骤复提1遍。合并2次提取液，真空旋蒸浓缩至原体积的1/10，加入4倍体积无水乙醇过夜醇沉。离心收集沉淀固体，即粗多糖提取物。对粗多糖提取物进行除杂，包括除蛋白、除脂肪和脱脂，经离子纯化及凝胶纯化得到精制的DOP。

1.3　实验动物分组及处理　将20只C57BL/6雌性小鼠（15月龄）随机分为老年组和老年鼠DOP处理组（DOP组），每组10只。另取10只雌性C57BL/6小鼠（5月龄）作为年轻组，小鼠选取依据参考文献［7-8］方法。3组小鼠每天均正常给水喂食。年轻组和老年组小鼠每日灌胃生理盐水1次，DOP组小鼠每日灌胃DOP，共持续2个月，直至小鼠为17月龄。本研究根据已发表的实验研究[9]结果，确定适宜的DOP剂量为200 mg·kg^{-1}·d^{-1}，灌胃0.2 mL。

1.4　各组小鼠体质量、摄食量和悬挂时间　每周记录各组小鼠体质量，连续记录8周的体质量。记录各组小鼠在灌胃第2个月（老年鼠为17月龄）时的每周平均摄食量和悬挂时间，连续监测4周。悬挂时间检查以小鼠笼盖的金属网格作为测试工具，笼盖的位置高于垫层15 cm，以防小鼠跌落及自主跳下笼盖。将小鼠放置于笼盖网格上，待其四肢抓牢后反转网格，使小鼠悬挂并启动计时，小鼠跌落后停止计时。

1.5　HE染色观察各组小鼠肠道和脾脏组织病理形态表现　取各组小鼠肠道和脾脏组织，采用4%多聚甲醛溶液进行固定，24 h后，梯度乙醇脱水，石蜡包埋后，切片至4 μm厚度，脱蜡，HE染色，于显微镜下观察拍照。

1.6　免疫组织化学染色检测各组小鼠肠道组织中ZO-1和MUC2蛋白表达水平　使用二甲苯和乙醇梯度脱蜡，3%过氧化氢浸泡使内源性过氧化物酶失活，PBS缓冲液冲洗切片3次，用5%正常山羊血清阻断非特异性结合；将切片与一抗（1∶300）在4 ℃下孵育过夜；用PBS缓冲液洗涤3次后，将组织切片与生物素标记的二抗室温孵育20 min后用氧化物酶链霉亲和素对切片进行染色，然后加入比色底物DAB，树胶封片，对蛋白进行定量分析。采用Image J软件分析阳性蛋白累积光密度（integrated option density，IOD），以IOD值表示蛋白表达水平。实验重复3次。

1.7　各组IBMM的制备　收集年轻组、老年组和DOP组小鼠的粪便，立刻投入液氮冷冻，并使用研钵粉碎（液氮少量多次加入）后装入50 mL离心管中；取粉末状粪便（120 mg）分别溶解于100 mL冷的M9溶液（线虫使用）和DMEM培养液（Caco-2细胞使用）中。并于4 ℃摇床上以200 r·min^{-1}的速度保持3 h，以便代谢物充分溶解。悬浮培养基依次用0.45 μm过滤器和0.22 μm注射器过滤2次（2次均为无菌操作），制成3组IBMM，其中*C. elegans* IBMM混合代谢物：OP50菌液为1∶10，Caco-2细胞IBMM混合代谢物：DMEM完全培养基为1∶40，各组分别以Young-IBMM、Aged-IBMM和DOP-IBMM表示，观察各组IBMM对*C. elegans*和Caco-2细胞的影响，制备方法参考文献［10］方法。

1.8　免疫荧光法检测各组*C. elegans*脂褐素累积水平和肠道渗漏情况　采用*C. elegans*生长培养基（nematode growth medium，NGM）培养*C. elegans*，以大肠杆菌（*Escherichia coli*，*E. coli*）OP50作为

食物来源。以混合代谢物：OP50菌液为1∶10的比例制备各组*C. elegans*的Young-IBMM、Aged-IBMM和DOP-IBMM，每组各50条。*C. elegans*同期化至L4期时，将Young-IBMM组*C. elegans*转至含年轻小鼠IBMM中，在成虫第1天进行脂褐素累积水平及肠道屏障完整性测定，以Young-IBMM组*C. elegans*为基准，Aged-IBMM组和DOP-IBMM组*C. elegans*在成虫第12天时进行后续脂褐素累积水平及肠道屏障完整性的测定。脂褐素的测定：用10 mmol·L^{-1}盐酸咪唑麻醉后，共聚焦荧光显微镜检测脂褐素颗粒积累情况。采用Image J软件分析脂褐素的荧光强度，结果表示为每个*C. elegans*的平均像素强度，以荧光强度代表脂褐素累积水平，脂褐素测定方法参考文献［11］。采用亮蓝染色法检测肠道屏障完整性，肠道渗漏情况结果以Young-IBMM组*C. elegans*亮蓝染料渗出情况为基准对照，观察Aged-IBMM组和DOP-IBMM组*C. elegans*肠道渗漏情况，亮蓝染色方法参考文献[12]方法。

1.9 Western blotting法检测Caco-2细胞中ZO-1、Occludin、TNF-α、IL-6和p-MLC/MLCK信号通路相关蛋白表达水平 以每孔1×10^5个的密度将Caco-2细胞接种于6孔细胞培养板中，培养24 h后，吸去培养液，以混合代谢物：DMEM为1∶40的比例制备Caco-2细胞条件培养基，培育48 h后进行后续实验。提取Caco-2细胞总蛋白，制备上清液。采用BCA法测定蛋白浓度，进行电泳、转膜，5% BSA封闭1 h后将膜与一抗ZO-1、Occludin、TNF-α、IL-6、p-MLC、MLC、MLCK和GAPDH于4 ℃条件下孵育过夜。用含0.05%Tween-20的TBST缓冲液洗涤3次（每次10 min）后与兔二抗在室温下孵育1 h[13]。用含有0.1%的TBST缓冲液洗膜后，用增强型化学发光剂（enhanced chemiluminescence，ECL）进行检测。采用Image J软件分析蛋白条带灰度值，以GAPDH为内参，计算目的蛋白表达水平。目的蛋白表达水平＝目的蛋白条带灰度值/内参蛋白条带灰度值。

1.10 统计学分析 采用SPSS 27.0统计软件进行统计学分析。各组小鼠体质量、摄食量和悬挂时间，小鼠肠道组织中ZO-1和MUC2蛋白表达水平，肠道脂褐素累积水平，Caco-2细胞中ZO-1、Occludin、TNF-α、IL-6和p-MLC/MLCK信号通路相关蛋白表达水平均符合正态分布，以$\bar{x}\pm s$表示，多组间样本均数比较采用单因素方差分析，组间两两比较采用LSD-*t*检验。以$P<0.05$为差异有统计学意义。

2 结 果

2.1 各组小鼠体质量、摄食量和悬挂时间 与年轻组比较，老年组小鼠体质量增加（$P<0.05$），摄食量降低（$P<0.05$），悬挂时间缩短（$P<0.05$）；与老年组比较，DOP组小鼠体质量明显降低（$P<0.01$），摄食量增加（$P<0.05$），悬挂时间明显延长（$P<0.01$）。见表1。

表1 各组小鼠体质量、摄食量和悬挂时间

Tab. 1 Body mass, food takes, hanging time of mice in various groups （$n=10, \bar{x}\pm s$）

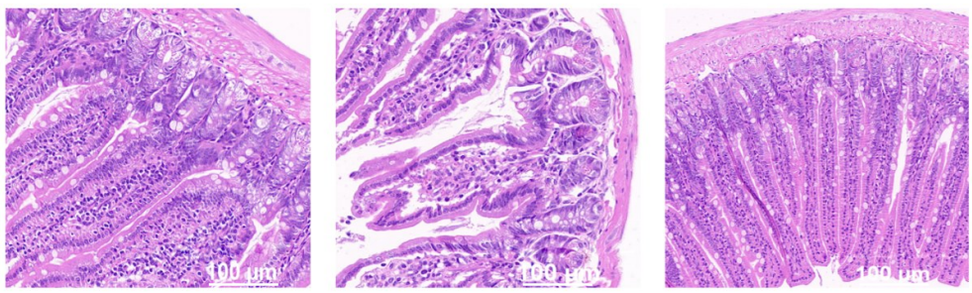
Group	Body mass(m/g)	Food take(m/g)	Hanging time(t/s)
Young	22.00±0.21	23.96±0.16	67.88±1.60
Aged	40.44±0.46*	17.78±0.19*	21.05±0.90*
DOP	36.95±0.87△△	19.99±0.37△	47.20±0.85△△

*$P<0.05$ compared with young group; △$P<0.05$, △△$P<0.01$ compared with aged group.

2.2 各组小鼠肠道和脾脏组织病理形态表现 年轻组小鼠肠黏膜上皮细胞结构完整，杯状细胞数量多，肠绒毛长度均匀，排列整齐，脾脏结构未见异常，未见明显生发中心；老年组小鼠肠黏膜厚度变薄，杯状细胞数量减少，肠绒毛长短不一且排列无序，脾脏表面可见大量铁血黄素存在，红髓内细胞成分减少，白髓内动脉周围淋巴鞘和淋巴小结残存或几乎消失；DOP组小鼠肠黏膜细胞结构和肠绒毛形态均得到明显改善，脾脏红髓整体功能改善，白髓成分增加。见图1和2。

2.3 各组小鼠肠道组织中ZO-1和MUC2蛋白表达水平 与年轻组比较，老年组小鼠肠道组织中ZO-1和MUC2蛋白表达水平降低（$P<0.05$或$P<0.001$）；与老年组比较，DOP组小鼠肠道组织中MUC2和ZO-1蛋白表达水平明显升高（$P<0.05$或$P<0.001$）。见图3、4和表2。

2.4 各组*C. elegans*脂褐素累积水平和肠道渗漏情况 与Young-IBMM组（1.00±0.01）比较，Aged-IBMM组*C. elegans*脂褐素累积水平（1.78±0.03）明显升高（$P<0.001$）；与Aged-IBMM组比较，DOP-IBMM组*C. elegans*脂褐素累积水平（0.70±0.09）明显降低（$P<0.001$）。与

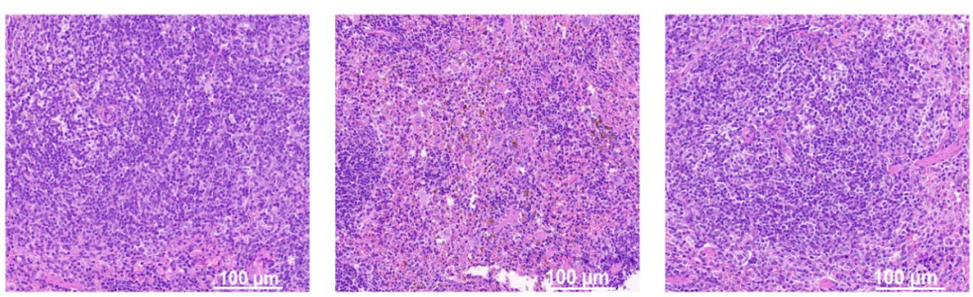


A　　B　　C

A：Young group；B：Aged group；C：DOP group.

图1　各组小鼠肠道组织病理形态表现(HE)

Fig. 1　Pathomorphology of intestinal tissue of mice in various groups(HE)

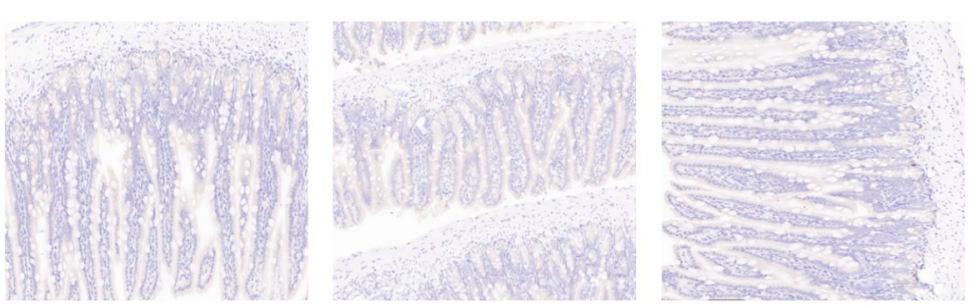


A　　B　　C

A：Young group；B：Aged group；C：DOP group.

图2　各组小鼠脾脏组织病理形态表现(HE)

Fig. 2　Pathomorphology of spleen tissue of mice in various groups(HE)



A　　B　　C

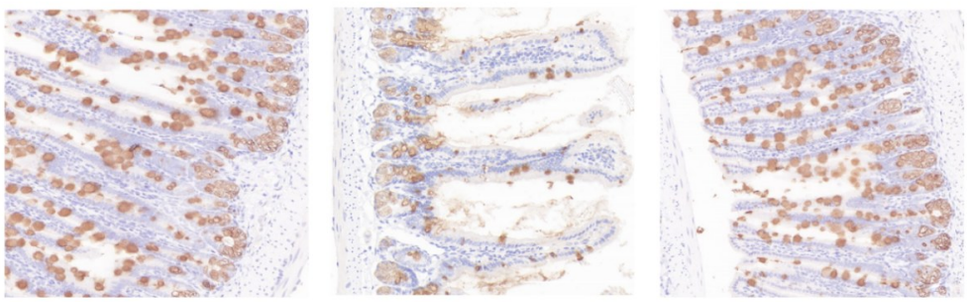
A：Young group；B：Aged group；C：DOP group.

图3　各组小鼠肠道组织中ZO-1蛋白表达情况(免疫组织化学，×100)

Fig. 3　Expressions of ZO-1 protein in intestinal tissue of mice in various groups (Immunohistochemistry，×100)

Young-IBMM组比较，Aged-IBMM组*C. elegans*中的亮蓝染料渗漏至线虫全身，使肠道结构模糊不清，不易观察；与Aged-IBMM组比较，DOP-IBMM组*C. elegans*亮蓝染料渗漏情况明显改善。见图5。

2.5　各组Caco-2细胞中TNF-α、IL-6、ZO-1和Occludin蛋白及p-MLC/MLCK信号通路相关蛋白表达水平　与Young-IBMM组比较，Aged-IBMM组Caco-2细胞中TNF-α、IL-6、p-MLC和MLCK蛋白表达水平明显升高（$P<0.05$、$P<0.01$或$P<0.001$），ZO-1和Occludin蛋白表达水平明显降低（$P<0.05$或$P<0.01$）；与Aged-IBMM组比较，DOP-IBMM组Caco-2细胞中TNF-α、IL-6、p-MLC和MLCK蛋白表达水平明显降低（$P<0.01$），ZO-1和Occludin蛋白表达水平明显升高（$P<0.05$或$P<0.01$）。见图6。



A　　B　　C

A：Young group；B：Aged group；C：DOP group.

图 4　各组小鼠肠道组织中 MUC2 蛋白表达情况（免疫组织化学，×100）

Fig. 4　Expressions of MUC2 protein in intestinal tissue of mice in various groups (Immunohistochemistry, ×100)

表 2　各组小鼠肠道组织中 ZO-1 和 MUC2 蛋白表达水平

Tab. 2　Expression levels of ZO-1 and MUC2 proteins in intestinal tissue of mice in various groups　（$n=3$, $\bar{x}\pm s$）

Group	ZO-1	MUC2
Young	0.13±0.01	0.25±0.01
Aged	0.08±0.06*	0.17±0.01**
DOP	0.16±0.07△	0.20±0.01△△

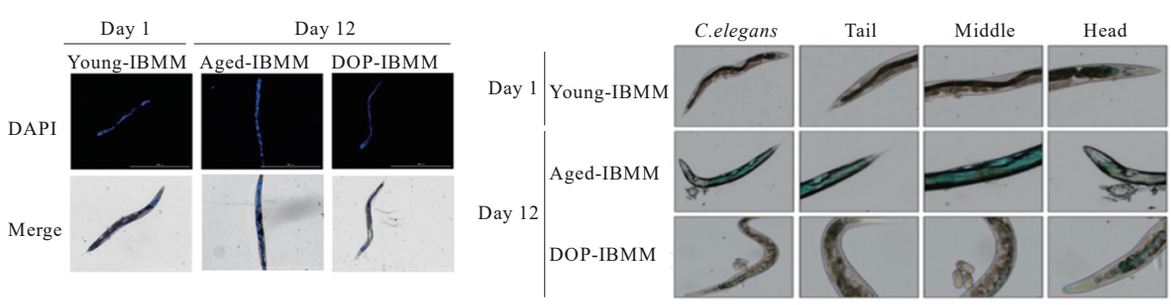
*$P<0.05$, **$P<0.001$ compared with young group; △$P<0.05$, △△$P<0.001$ compared with aged group.

3　讨　论

衰老是引起肠道微生态失调的主要危险因素之一。利用中药调控肠道微生态失调改善肠道黏膜损伤是延缓衰老进程的一个直接策略。研究[14]显示：C57BL/6 小鼠 3～6 月龄为年轻期，10～14 月龄为中年期，15～24 月龄为老年期，且随着月龄的增长，老年期小鼠行为特征发生改变，包括体质量增加、摄食量减少和悬挂时间缩短等。本研究结果显示：DOP 可降低老年小鼠体质量、增加摄食量并明显延长悬挂时间，表明 DOP 可改善老年小鼠生理指标，发挥延缓衰老的作用。

衰老发生常伴随着肠道黏膜细胞结构紊乱导致的屏障损伤和通透性增加，进而使抗原、内毒素和病原体等促炎因子进入血液及淋巴循环，导致脾脏结构紊乱，免疫功能降低等[15-16]。研究[17-18]显示：老年肠道屏障功能损伤与 ZO-1 和 MUC2 蛋白在老年肠道中的表达水平明显降低存在密切关联。本研究结果显示：DOP 干预后，老年小鼠小肠黏膜细胞结构和肠绒毛形态均得到明显改善，脾脏红髓整体功能改善，白髓成分增加；DOP 可有效提高老年小鼠肠道内 ZO-1 和 MUC2 蛋白表达水平。提示 DOP 可通过改善肠道屏障损伤延缓衰老。

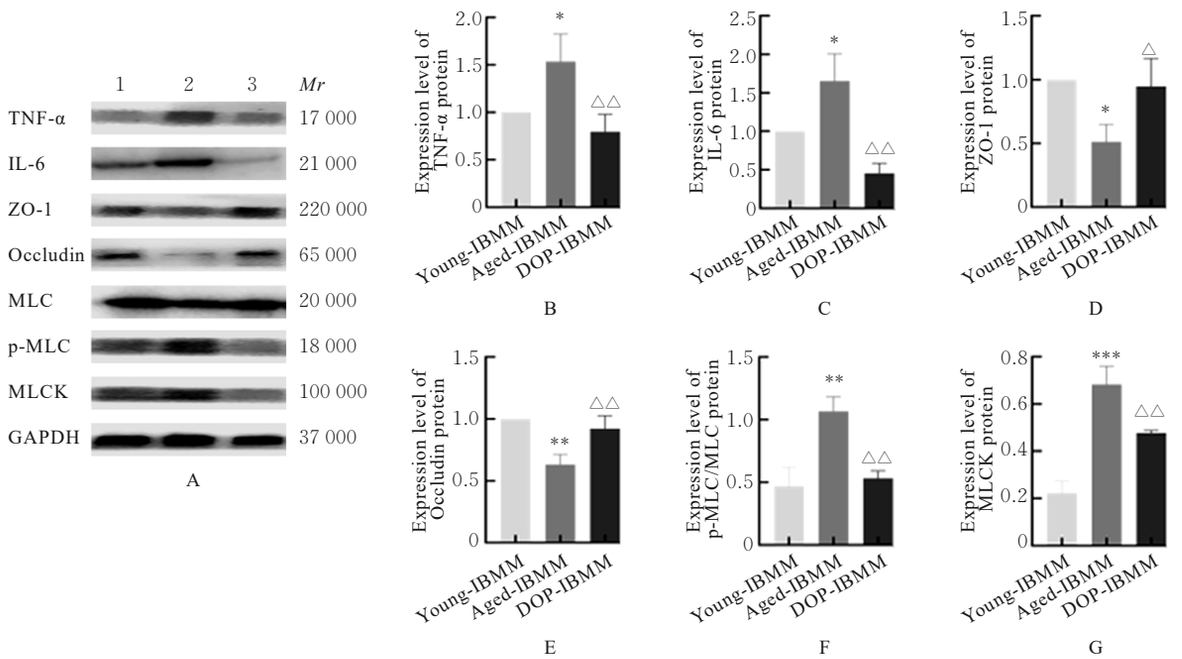
老年肠道屏障功能障碍的关键始动因素为肠道菌群代谢紊乱，从而导致有益代谢物减少，而有害代谢物增加[19-21]。本研究建立 IBMM 体系，以 *C. elegans* 为模型，检测衰老指数以及屏障相关指标，进一步明确 DOP 的屏障修复功能与肠菌代谢途径存在关联。研究[22-23]显示：脂褐素为 *C. elegans* 肠道脂质氧化的代谢产物，随着年龄的增长脂褐素会在线虫肠道内不断积累，可作为 *C. elegans* 衰老的生物标志物。亮蓝染料与 *C. elegans* 食用菌混合后会随 *C. elegans* 进食进入 *C. elegans* 体内并停留在肠道内，因此，



A　　B

图 5　各组 *C.elegans* 脂褐素累积情况（A）和肠道渗漏情况（B）

Fig. 5　Lipofuscin accumulation (A) and intestinal leakage (B) of *C.elegans* in various groups



Lane 1: Young-IBMM group; Lane 2: Aged-IBMM group; Lane 3: DOP-IBMM group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared with Young-IBMM group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$ compared with Aged-IBMM group.

图6　Western blotting法检测各组Caco-2细胞中肠屏障相关蛋白表达电泳图(A)和直条图(B～G)

Fig.6　Electrophoregram(A) and histograms(B—G) of expressions of intestinal barrier-related proteins in Caco-2 cells in various groups detected by Western blotting method

若*C. elegans*肠道屏障完整，亮蓝染色拍照后会留下完整*C. elegans*肠道形态，若*C. elegans*肠道发生渗漏，亮蓝染料会弥漫至*C. elegans*全身。本研究结果显示：DOP的IBMM干预后*C. elegans*脂褐素体内累积水平明显降低，亮蓝染色显示*C. elegans*肠道渗漏情况得到明显改善，提示DOP可以通过提高肠菌互作产生有益代谢物，抑制肠道屏障损伤从而发挥延缓衰老的功效。

研究[24-25]表明：肠道屏障的破坏是由MLC磷酸化引起的，MLC被MLCK激活，从而导致紧密连接通透性增加。而MLCK1作为肠道屏障功能的主要调节因子，会在炎症诱导下表达增加并转运至肌动球蛋白环，从而使MLC发生磷酸化并诱导Occludin内吞，最终导致细胞骨架重构，细胞间通透性增加，肠道屏障功能丧失。本研究结果显示：Aged-IBMM组Caco-2细胞中促炎因子TNF-α和IL-6表达水平明显升高，导致上皮屏障损伤和功能障碍，与既往研究[26]结论一致。DOP-IBMM组p-MLC/MLCK信号通路转导受到抑制，炎症因子释放减少，肠道组织中ZO-1和Occludin等紧密连接蛋白表达水平升高，老年小鼠肠道屏障损伤得到改善。

综上所述，DOP可以有效提高老年小鼠生理指标，改善肠道黏膜细胞和脾脏结构紊乱，恢复肠道屏障损伤；其机制可能是通过改善肠道细菌代谢物，抑制p-MLC/MLCK信号通路介导的促炎因子分泌，提高老年小鼠肠道紧密连接蛋白表达，从而发挥对老年小鼠肠道屏障损伤的保护作用。

利益冲突声明：

所有作者声明不存在利益冲突。

作者贡献声明：

李宏参与实验设计、论文撰写和修改，王慧、王隶书和王超楠参与实验数据的获取及分析，徐晓浩和孙立伟参与指导论文撰写。

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