

有氧运动对APP/PS1小鼠肠黏膜屏障功能和焦虑样行为的影响

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摘要 该研究旨在探究有氧运动对APP/PS1转基因AD小鼠肠黏膜屏障功能、焦虑样行为和TLR4/NF- κ B信号通路的影响。将APP/PS1双转基因小鼠随机分为安静组(SE-AD)、有氧运动组(EX-AD), C57BL/6J小鼠作为对照(WT)。运动组小鼠进行12周有氧运动干预后, 采用旷场实验评估其焦虑样行为; ELISA检测血浆DAO、D-LA、LPS含量; HE染色观察结肠形态; 免疫荧光检测结肠ZO-1、Occludin蛋白表达水平; Western blot检测结肠紧密连接蛋白、TLR4/MyD88/NF- κ B信号通路相关蛋白和炎症因子蛋白表达水平。结果显示, 有氧运动使AD小鼠在旷场中移动的距离显著性缩短($P<0.01$), 在旷场中心区域移动的时间、移动的路程百分比均显著性增加($P<0.05$, $P<0.01$); 有氧运动使AD小鼠血浆DAO、D-LA、LPS含量显著性下降($P<0.05$, $P<0.01$); SE-AD组小鼠结肠细胞核较少, 黏膜层较薄, 疏松多孔, 黏膜上皮存在破损, 柱状细胞较少且排列不规则; EX-AD组小鼠结肠细胞核增多, 黏膜柱状细胞排列规则, 黏膜上皮较厚; 有氧运动显著增加AD小鼠结肠ZO-1、Occludin荧光面积($P<0.01$), 上调Claudin-1、ZO-1、Occludin蛋白表达水平($P<0.05$, $P<0.01$), 下调TLR4、MyD88、NF- κ B、TNF- α 、IL-1 β 蛋白表达水平($P<0.05$, $P<0.01$)。这表明有氧运动可改善AD小鼠肠黏膜屏障功能, 减少焦虑样行为, 其作用机制与上调肠道紧密连接蛋白水平, 弱化LPS易位, 抑制TLR4/MyD88/NF- κ B信号通路减少炎症因子释放有关。

关键词 阿尔茨海默病; 有氧运动; 焦虑样行为; 肠道屏障; 紧密连接蛋白; LPS位移

Effects of Aerobic Exercise on Intestinal Mucosal Barrier Function and Anxiety-Like Behavior in APP/PS1 Mice

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Abstract This study aimed to explore the effects of aerobic exercise on intestinal mucosal barrier function, anxiety-like behavior, and the TLR4/NF- κ B signaling pathway in APP/PS1 transgenic AD (Alzheimer's disease) mouse models. APP/PS1 double transgenic mice were randomly divided into the sedentary group (SE-AD) and the aerobic exercise group (EX-AD), while C57BL/6J mice were raised as the control group (WT). After 12 weeks of

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aerobic exercise intervention in the mice of the EX-AD group, the open field test was used to evaluate anxiety-like behavior. ELISA was used to measure plasma levels of DAO, D-LA, and LPS. Histological analysis of the colon was performed with HE (hematoxylin-eosin) staining. Immunofluorescence was used to detect ZO-1 and Occludin in the colon. Western blot was performed to determine the expression levels of tight junction proteins, TLR4/MyD88/NF- κ B pathway-associated proteins, as well as inflammatory cytokines in the colon. The results showed that aerobic exercise significantly reduced the distance of AD mice in the open field ($P<0.01$), and significantly increased not only the time spent moving in the central area, but also the percentage of distance traveled ($P<0.05$, $P<0.01$). Furthermore, aerobic exercise significantly decreased the plasma levels of DAO, D-LA, and LPS in AD mice ($P<0.05$, $P<0.01$). In the SE-AD group, the colon cells had fewer nuclei, a thinner mucosal layer which was loose and porous with epithelial damage, and the columnar cells were fewer and arranged irregularly. In contrast, the EX-AD group showed more colonic cell nuclei, regular arrangement of mucosal columnar cells, and thicker mucosal epithelium. Aerobic exercise significantly increased the fluorescence area of ZO-1 and Occludin in the colon of AD mice ($P<0.01$), up-regulated the expression levels of Claudin-1, ZO-1 and Occludin ($P<0.05$, $P<0.01$), and down-regulated the expression of TLR4, MyD88, NF- κ B, TNF- α , and IL-1 β ($P<0.05$, $P<0.01$). These results suggest that aerobic exercise can improve the intestinal mucosal barrier function and alleviate anxiety-like behavior of AD mice. Its mechanism of action is related to the up-regulation of intestinal tight junction proteins, the reduction of LPS displacement, and the inhibition of the TLR4/MyD88/NF- κ B signaling pathway, which in turn reduces the release of inflammatory factors.

Keywords Alzheimer's disease; aerobic exercise; anxiety-like behaviour; intestinal barrier; tight junction protein; LPS displacement

阿尔茨海默病(Alzheimer's disease, AD)是最常见的神经退行性疾病,其特征是记忆能力和各种认知功能的进行性下降^[1]。随着病情的发展,患者可能会出现语言障碍和空间意识障碍。这些临床症状通常还伴随着神经精神症状,如焦虑、冷漠、痴迷、抑郁和精神紊乱^[1-2]。世界卫生组织估计,AD影响全球超过5 520万人,每年报告出1 000万新病例^[3]。预测表明,到2030年,受影响人数将达到8 200万,到2050年可能增加到1.52亿,对公共卫生构成重大挑战^[3]。因此,迫切需要对AD进行深入研究,以解决这一对人类健康构成重大威胁的疾病。最近的研究表明,传统的AD治疗对暂时缓解疾病的进展十分有限,包括Aducanumab在内的6项药物III期临床试验均未能取得理想的结果^[4-5]。因此,研究AD的发病机制,探究新的药物或者干预手段已成为当前研究的焦点。

肠道屏障通过促进营养吸收和防止病原体进入,在健康和疾病中起着至关重要的作用^[6]。LEBLHUBER等^[7]发现,AD患者粪便中钙卫蛋白水平升高,表明存在肠道屏障泄漏。HESTON等^[8]的队列研究发现,即使在AD早期阶段,肠道炎症也与脑部病理有关,肠道屏障破坏和炎症可能会加剧AD的进

展。ANDREADOU等^[9]发现,AD患者血清和脑脊液中细菌脂多糖(lipopolysaccharide, LPS)升高。LPS是肠道革兰氏阴性菌表面的成分,进入人体或大脑,可以诱发炎症加剧AD病理^[10]。以上表明,AD存在肠道屏障功能损害,如何调节肠道屏障功能可能是减缓AD的有效途径。

此外,近年来发现,90%的AD患者会出现抑郁和焦虑等神经精神症状^[11],65%的AD患者在患病期间会出现严重的精神症状^[12]。而过量的LPS位移到内环境,已被证实可诱导抑郁和焦虑行为^[13-14]。但目前对AD焦虑抑郁相关的研究还十分有限。越来越多的临床证据表明,运动作为一种非药物方法,对预防和治疗老年人认知能力下降具有积极作用^[15-16]。但运动对AD肠道黏膜屏障功能和神经精神症状的影响作用目前尚不明确。APP/PS1双转基因小鼠表达嵌合小鼠/人淀粉样蛋白前体蛋白(Mo/HuAPP695swe)和突变人早老蛋白1(PS1-dE9)基因,可出现早发性AD,是目前国际上公认研究AD较为理想和经典的模型动物^[17]。本研究采用APP/PS1转基因AD小鼠进行有氧运动干预,确认运动对AD小鼠肠道屏障功能和焦虑样行为的影响,并探讨TLR4/NF- κ B信号通路调控机制,以期为AD的预防和康复提供实验依据。

1 材料与方法

1.1 实验动物及分组

本实验选取3月龄SPF级雄性APP/PS1双转基因小鼠20只及同龄雄性C57BL/6J小鼠10只,均由常州卡文斯实验动物有限公司提供[许可证号:SCXK(苏)2016-0010]。实验中,APP/PS1小鼠被随机分为安静组(SE-AD)和有氧运动组(EX-AD),而C57BL/6J小鼠则作为野生型安静对照组(WT)。所有小鼠均饲养在通风良好且密闭的塑料笼内,提供自由摄食和饮水,并维持12 h光照与12 h黑暗的昼夜交替环境,温度保持在22 °C至25 °C。本实验严格遵循福建师范大学动物伦理委员会的指导原则,并获得福建师范大学动物伦理委员会的批准(审批号:20241012)。

1.2 主要实验试剂

4%多聚甲醛购于北京索莱宝科技有限公司。二氨氧化酶(diamine oxidase, DAO)(RX201098M)、D-乳酸(d-lactic acid, D-LA)(JRX200596)和LPS(JRX2025356) ELISA试剂盒购自泉州睿信生物科技有限公司。Claudin-1(28674-1-AP)、ZO-1(21773-1-AP)、Occludin(27260-1-AP)、TLR4(19811-1-AP)、MyD88(67969-1-Ig)、NF- κ B(10745-1-AP)、TNF- α (17590-1-AP)、IL-1 β (26048-1-AP)、山羊抗兔重组二抗(SA00001-2)均购自武汉三鹰生物技术有限公司。苏木精-伊红染液(G1005-100ML)、RIPA裂解液(G2002-100ML)、cocktail蛋白酶抑制剂(G2006-250UL)、BCA蛋白质浓度测定试剂盒(G2026-200T)、ECL发光试剂盒(G2161-200ML)均购自武汉赛维尔生物科技有限公司。

1.3 主要实验仪器

动物实验跑台购自安徽正华生物仪器设备有限公司。高速冷冻离心机购自广州吉迪仪器有限公司。酶标仪购自上海科华实验系统有限公司。切片机购自浙江益迪医疗设备公司。垂直电泳仪购自北京六一生物科技有限公司。倒置荧光显微镜购自德国Leica公司。Bio-Rad ChemiDoc XRS+化学发光凝胶成像系统购自美国Bio-Rad公司。

1.4 运动方案

参考跑速与氧耗量的研究^[18],本实验设计了一种为期12周的跑台递增强度运动方案,用于小鼠的运动干预。在该方案中,EX-AD组小鼠接受跑台训练,运动强度从第1周至第8周逐步递增,速度从7 m/min逐渐提升至14 m/min;第9周至第12周则保持15 m/min的

速度进行恒定训练。根据SCHEFER等^[19]的研究,该运动负荷强度属于中等强度有氧运动范畴。运动频率为每周5次,每次持续45 min。与此同时,WT组和SE-AD组小鼠在12周内维持常规静息生活方式。在EX-AD组进行跑台运动期间,WT组和SE-AD组小鼠被放置于静止的跑台上,以确保环境因素的一致性。

1.5 旷场实验

最后一次运动训练结束后,第二天每组随机选取6只小鼠,在实验开始时,将小鼠放置在长和宽均为40 cm、高为50 cm的开放实验箱正中间,旷场顶端的红外摄像监控录制小鼠5 min内的自由活动。记录小鼠在旷场内活动的总路程、在中央区域的活动时间和路程,以此来评估小鼠的焦虑样行为。

1.6 取材

在完成旷场实验后,小鼠禁食12 h,其间允许其自由饮水。次日采用异氟烷对小鼠进行麻醉,随后通过注射器进行心脏采血。采集的血液在4 °C、3 000 r/min条件下离心20 min,分离上清液,并将其置于-80 °C冰箱中保存,以备后续的ELISA检测。与此同时,将小鼠结肠组织迅速放入冻存管,并立即投入液氮中,之后将其转移至-80 °C冰箱保存,用于后续的Western blot实验。此外,每组随机选取4只小鼠,通过多聚甲醛进行心脏灌注,直至肝脏呈现白色。随后,取出结肠组织并将其置于4%多聚甲醛溶液中室温固定48 h,之后制作石蜡切片,用于HE染色及免疫荧光检测。

1.7 HE染色

对固定后的结肠组织进行常规处理,依次完成脱水、透明化、浸蜡和包埋步骤,制备厚度为5 μ m的切片。随后,将切片进行二甲苯脱蜡和经梯度乙醇水化处理,苏木素室温染色8 min,伊红室温染色45 s。染色完成后,切片经梯度乙醇脱水和二甲苯透明处理,最后使用中性树胶进行封片。

1.8 ELISA检测

首先将血浆样本按DAO、D-LA和LPS试剂盒要求稀释并加入预包被抗体的酶标板中。同时设置标准品和空白孔。1% BSA 37 °C孵育2 h后,加入酶标抗体继续37 °C孵育1 h,随后加入底物显色并终止反应。最后,通过酶标仪在450 nm波长下测定吸光度(D)值,结合标准曲线计算样本中DAO、D-LA和LPS的浓度。整个过程严格按照试剂盒说明书操作。

1.9 免疫荧光检测

取小鼠结肠组织石蜡切片,依次进行脱蜡、复水及抗原修复处理。将载玻片置于PBS中洗涤3次,去除多余溶液后,将切片浸入自发荧光淬灭剂中浸泡5 min,随后用流水冲洗。接着滴加BSA溶液,室温孵育20 min以封闭非特异性结合位点。之后,切片分别加入稀释比例为1:500的ZO-1和Occludin一抗,于4℃孵育过夜。次日,避光条件下加入稀释比例为1:1 000的二抗,室温孵育1 h。孵育结束后,用PBS清洗切片以去除多余的二抗,随后将切片置于干燥箱内低温烘干。最后,采用含DAPI的封片剂进行封片处理。在荧光显微镜下观察并采集图像,利用ImageJ软件对荧光面积进行定量分析。

1.10 Western blot

取各组小鼠结肠组织,按照组织重量与RIPA裂解液1:10的比例加入裂解液,并按10:1的比例加入蛋白酶抑制剂cocktail,随后进行匀浆处理并在4℃、12 000 r/min条件下离心15 min,收集上清液。接着,利用BCA蛋白质浓度测定试剂盒,按照说明书操作对蛋白样本进行定量分析。之后,依次进行SDS-PAGE电泳、转膜、封闭处理,随后进行一抗孵育。一抗包括Claudin-1(1:1 000)、ZO-1(1:5 000)、Occludin(1:5 000)、TLR4(1:1 000)、MyD88(1:2 000)、NF- κ B(1:2 000)、TNF- α (1:500)、IL-1 β (1:1 000)和 β -actin(1:50 000),孵育条件为4℃过夜。次日,用PBST洗涤膜3次,每次10 min,随后加入稀释比例为1:5 000的二抗,于室温摇床孵育1 h。再次用PBST洗涤3次,每次10 min,最后使用ECL发光试剂盒在Bio-Rad ChemiDoc XRS+化学发光凝胶成像系统中曝光成像。通过ImageJ软件分析条带的积分灰度值,以统计各蛋白的相对表达量。

1.11 统计学分析

所有数据以均值 $\pm$ 标准差($\bar{x}\pm s$)的形式呈现。统计分析和图表绘制采用GraphPad Prism 8.0软件完成。实验数据的统计学处理采用单因素方差分析,事后多重比较采用最小显著差异法,以 P 值小于0.05作为显著性差异的标准。

2 结果

2.1 有氧运动对AD小鼠焦虑样行为的影响

旷场实验结果(图1)显示,与WT组相比,SE-AD组小鼠在旷场中移动的距离显著性增加($P<0.01$);

与SE-AD组相比,EX-AD组小鼠在旷场中移动的距离显著性缩短($P<0.01$)(图1A)。与WT组相比,SE-AD组小鼠在旷场中心区域移动的时间、移动的路程百分比均显著性缩短($P<0.05$);与SE-AD组相比,EX-AD组小鼠在旷场中心区域移动的时间、移动的路程百分比显著性增加($P<0.05$ 或 $P<0.01$)(图1B和图1C)。如图1D所示,WT组小鼠在旷场中移动的轨迹较为分散,而SE-AD组移动路程增加,且较多集中在旷场边缘,EX-AD组移动距离缩短,且更多地向中央区域集中。以上结果表明,AD小鼠存在显著焦虑样行为,在有氧运动后得到了减轻。

2.2 有氧运动对AD小鼠血浆肠道屏障功能相关指标的影响

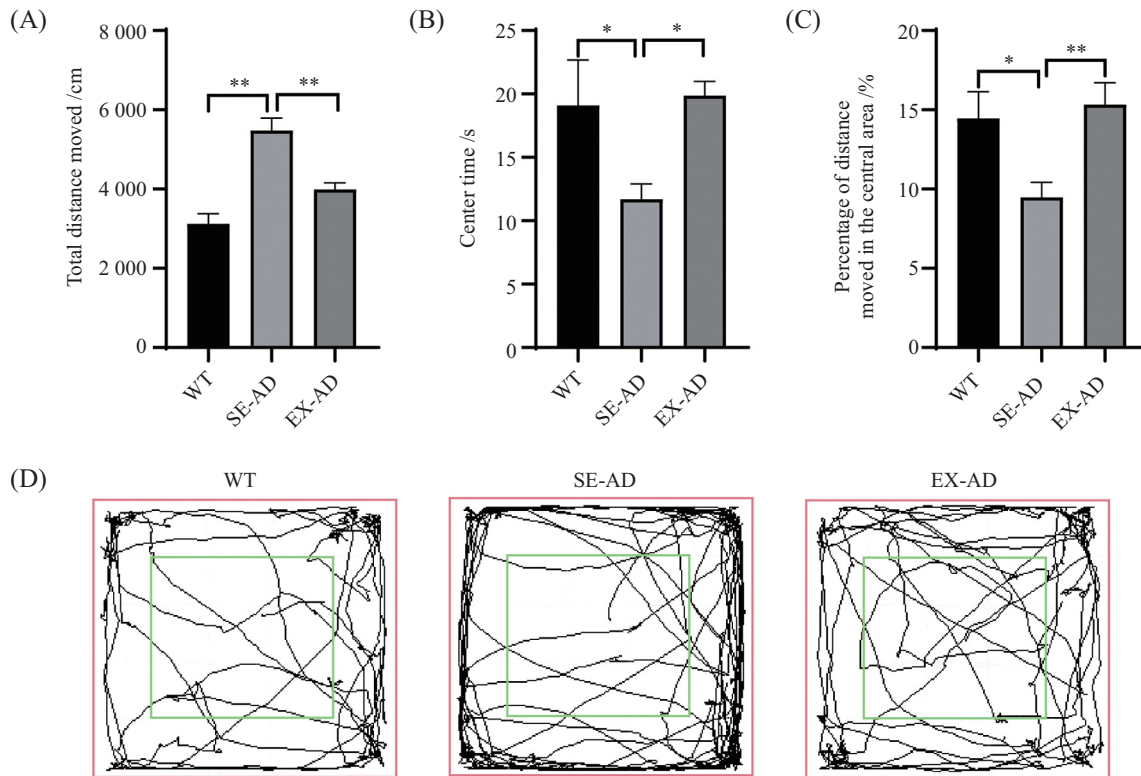
采用ELISA检测小鼠血浆肠道屏障功能相关指标的表达水平,如图2所示,与WT组相比,SE-AD组小鼠血浆DAO、D-LA、LPS含量显著性上升($P<0.01$);与SE-AD组相比,EX-AD组小鼠血浆DAO、D-LA、LPS含量显著性下降($P<0.05$ 或 $P<0.01$)。以上结果说明,AD小鼠肠道屏障功能下降,有氧运动可提高AD小鼠肠道屏障功能。

2.3 有氧运动对AD小鼠结肠病理学形态的影响

进一步采用HE染色观察小鼠结肠形态,如图3所示,WT组小鼠结肠细胞核密集,肠黏膜光滑完整,黏膜层上皮较厚,柱状细胞排列整齐致密;而SE-AD组小鼠结肠细胞核较少,黏膜层较薄,疏松多孔,黏膜上皮存在如图黑色箭头所示的破损,柱状细胞较少且排列不规则;EX-AD组小鼠结肠细胞核增多,黏膜柱状细胞排列规则,黏膜上皮较厚。这表明有氧运动可减轻AD小鼠肠黏膜损伤。

2.4 有氧运动对AD小鼠结肠紧密连接蛋白表达的影响

采用免疫荧光进一步对小鼠结肠ZO-1、Occludin进行检测,如图4所示,与WT组相比,SE-AD组小鼠结肠ZO-1、Occludin荧光面积显著性减小($P<0.05$ 或 $P<0.01$);与SE-AD组相比,EX-AD组小鼠结肠ZO-1、Occludin荧光面积显著性增加($P<0.01$)。定位在黏膜上皮的ZO-1与图3的病理变化一致。进一步采用Western blot检测结肠Claudin-1、ZO-1、Occludin的表达情况,如图5所示,与WT组相比,SE-AD组小鼠结肠Claudin-1、ZO-1、Occludin蛋白表达水平显著性下降($P<0.05$);与SE-AD组相比,EX-AD组小鼠结肠Claudin-1、ZO-1、Occludin蛋白表达水平

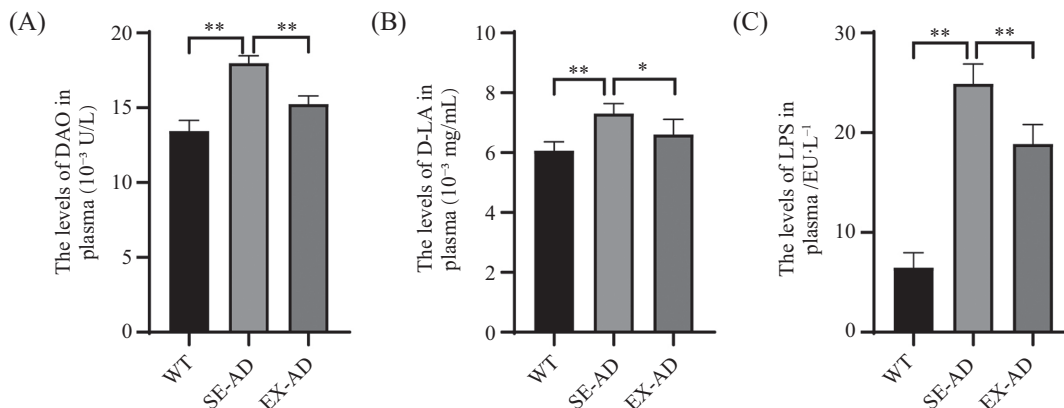


A: 小鼠在旷场中移动总距离; B: 小鼠在旷场中央区域移动的时间; C: 小鼠在旷场中央区域移动的路程比; D: 各组小鼠在旷场中移动轨迹代表图。数据以 $\bar{x} \pm s$ 表示, $n=6$, * $P<0.05$, ** $P<0.01$ 。

A: total distance moved by the mice in the open field; B: time moved by the mice in the central area of the open field; C: ratio of the distance traveled by the mice in the central area of the open field; D: graph of the trajectories of the mice in the open field in each group. Data are expressed as $\bar{x} \pm s$, $n=6$, * $P<0.05$, ** $P<0.01$.

图1 小鼠旷场实验结果

Fig.1 Results of the mouse open field experiment



A: 血浆DAO含量; B: 血浆D-LA含量; C: 血浆LPS含量。数据以 $\bar{x} \pm s$ 表示, $n=6$, * $P<0.05$, ** $P<0.01$ 。

A: DAO level in plasma; B: D-LA level in plasma; C: LPS level in plasma. Data are expressed as $\bar{x} \pm s$, $n=6$, * $P<0.05$, ** $P<0.01$.

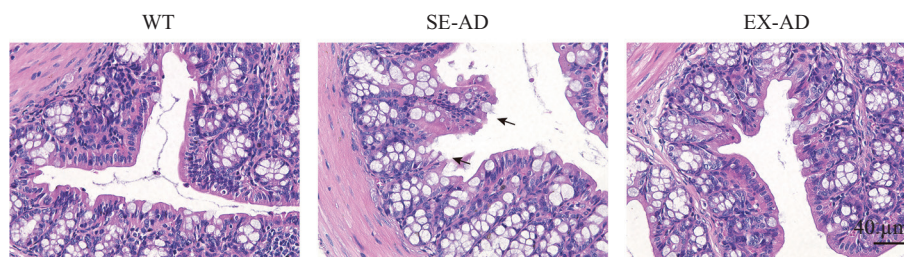
图2 小鼠血浆肠道屏障功能相关指标表达水平

Fig.2 Expression levels of plasma intestinal barrier function-related indicators in mice

显著性上升($P<0.05$ 或 $P<0.01$)。以上结果说明, 有氧运动可提高AD小鼠结肠紧密连接蛋白的表达水平。

2.5 有氧运动对AD小鼠结肠TLR4/MyD88/NF- κ B信号通路相关蛋白和炎症因子蛋白表达的影响

如图6所示, 与WT组相比, SE-AD组小鼠结

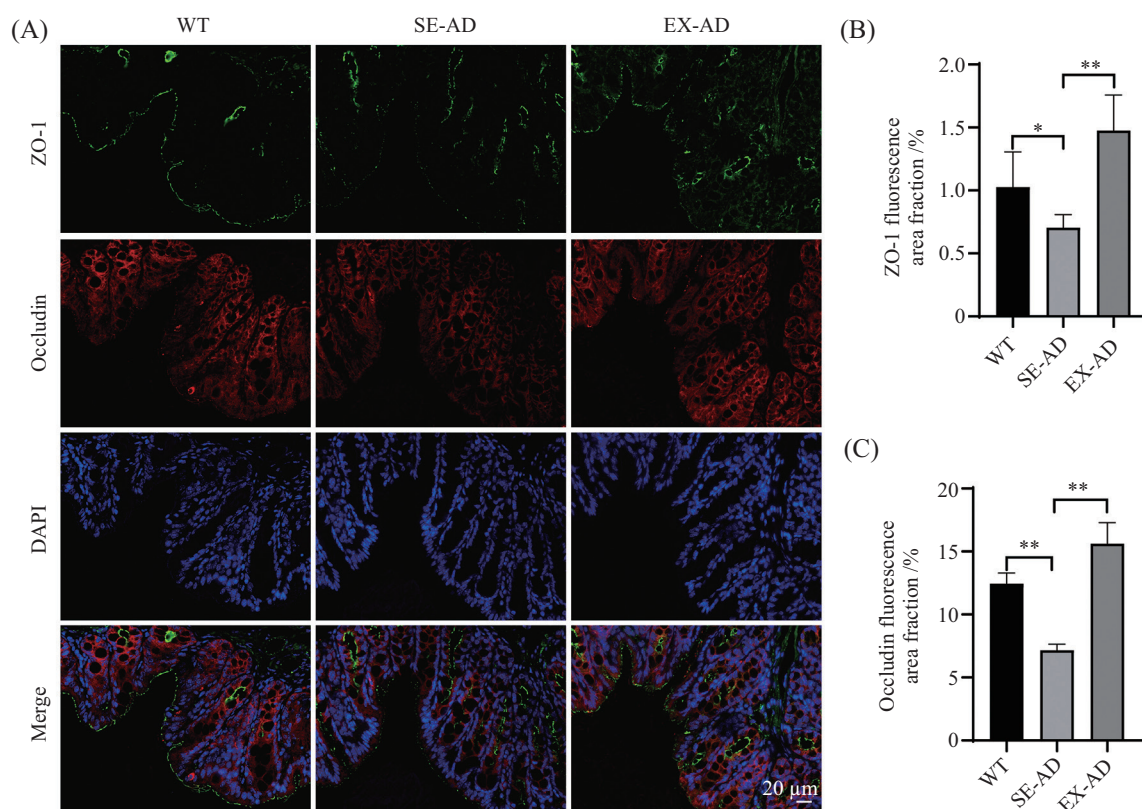


黑色箭头表示结肠黏膜上皮破损处。

The black arrow indicates the site of epithelial damage in the colonic mucosa.

图3 小鼠结肠HE染色

Fig.3 HE staining of mouse colon



A: 小鼠结肠ZO-1、Occludin免疫荧光代表图像; B: 小鼠结肠ZO-1荧光面积百分比; C: 小鼠结肠Occludin荧光面积百分比。数据以 $\bar{x} \pm s$ 表示, $n=4$, $*P<0.05$, $**P<0.01$ 。

A: representative images of mice colon ZO-1 and Occludin immunofluorescence; B: mice colon ZO-1 fluorescence area; C: mice colon Occludin fluorescence area. Data are expressed as $\bar{x} \pm s$, $n=4$, $*P<0.05$, $**P<0.01$.

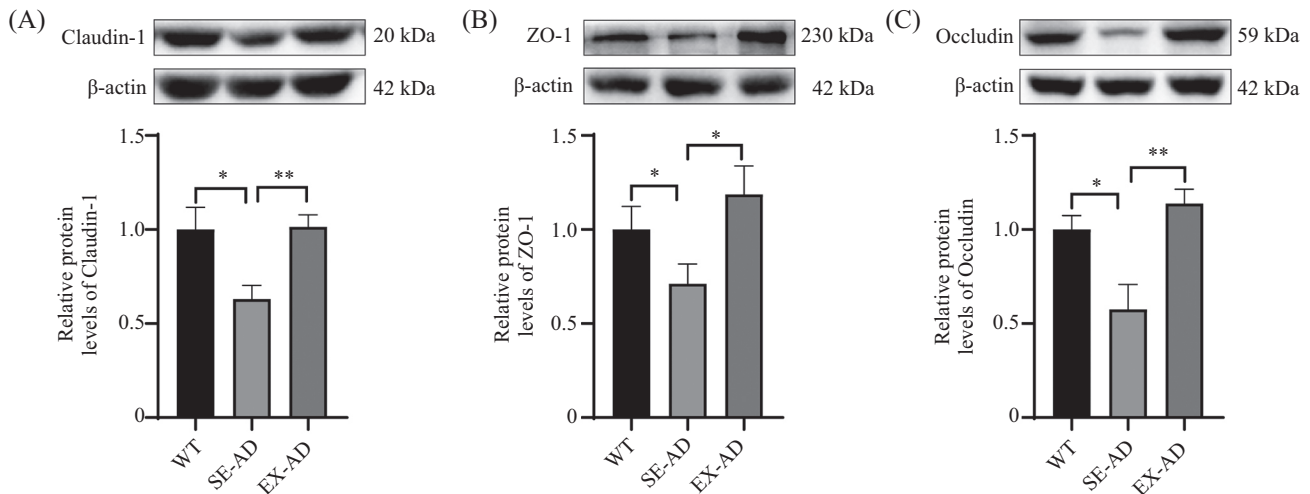
图4 小鼠结肠紧密连接蛋白免疫荧光

Fig.4 Immunofluorescence of mouse colonic tight junction proteins

肠TLR4、MyD88、NF- κ B蛋白水平显著性升高, 下游TNF- α 、IL-1 β 蛋白水平显著性上升 ($P<0.05$ 或 $P<0.01$); 与SE-AD组相比, EX-AD组小鼠结肠TLR4、MyD88、NF- κ B蛋白水平显著性降低, 下游TNF- α 、IL-1 β 蛋白水平显著性下降 ($P<0.05$ 或 $P<0.01$)。以上结果表明, 有氧运动可通过抑制TLR4/MyD88/NF- κ B信号通路降低AD小鼠结肠炎症因子的水平。

3 讨论

AD虽然临床症状表现为记忆的衰退, 但近年来发现, AD患者较多表现出焦虑和抑郁等神经精神症状, 并且在轻度认知障碍(mild cognitive impairment, MCI)阶段之前就已经出现^[20-21]。LI等^[22]的荟萃分析结果表明, 焦虑会增加MCI患者发展为AD的风险。此外, GALLAGHER等^[23]发现表现出高度焦虑的个体患AD的风险可增加48%, 并且从MCI转化为AD的



A: 小鼠结肠Claudin-1蛋白相对表达量; B: 小鼠结肠ZO-1蛋白相对表达量; C: 小鼠结肠Occludin蛋白相对表达量; 数据以 $\bar{x} \pm s$ 表示, $n=5$, * $P<0.05$, ** $P<0.01$ 。

A: relative expression of mice colonic Claudin-1 protein; B: relative expression of mice colonic ZO-1 protein; C: relative expression of mice colonic Occludin protein; data are expressed as $\bar{x} \pm s$, $n=5$, * $P<0.05$, ** $P<0.01$ 。

图5 小鼠结肠紧密连接蛋白表达水平

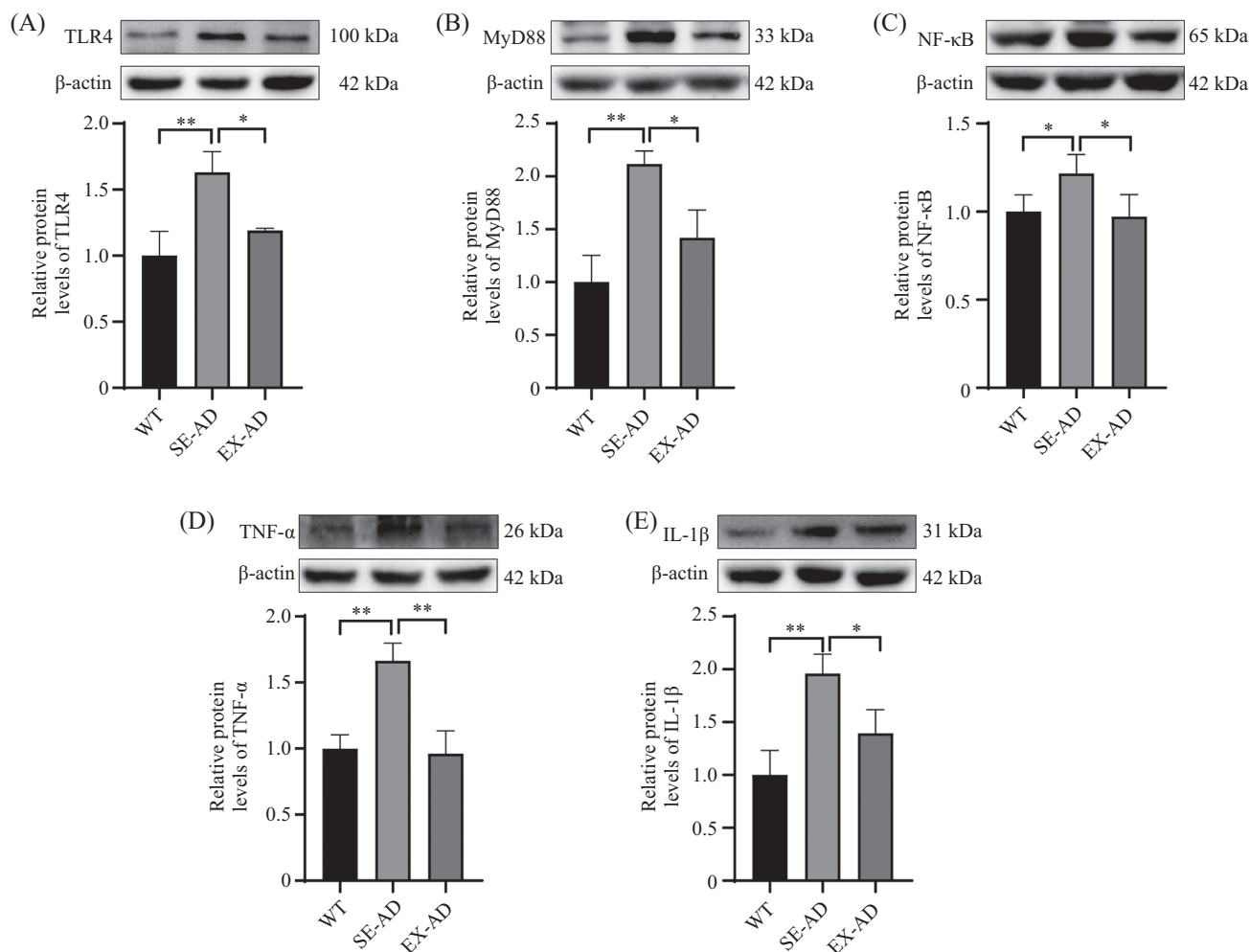
Fig.5 Expression levels of mouse colonic tight junction proteins

速度更快。鉴于AD神经精神症状的高患病率, 对其进行评估和治疗显得尤为重要。本实验采用旷场实验来观察AD小鼠的神经精神状态。结果发现, AD小鼠在旷场中移动的距离增加, 且较为集中在边缘区域活动, 在旷场中央区域运动的路程和时间都显著低于野生型小鼠, 而运动干预后在旷场中央区域活动的时间均得到了显著性提高。焦虑的动物在旷场中一般表现为运动距离缩短, 而SE-AD组小鼠在旷场中移动的距离显著增加, 这可能与AD小鼠兴奋性高有关, 兴奋性高的小鼠在旷场中通常表现出更高的运动活动水平^[24]。在啮齿动物中, 焦虑样行为被定义为通过暴露于潜在和/或模棱两可的威胁而引发的动机行为(即防御行为)^[25], 因此, 在旷场中焦虑程度高的小鼠更倾向于在旷场的边缘和角落运动。对小鼠进行抗焦虑药物的研究已经证实, 抗焦虑药物可增加小鼠在旷场中心区停留的时间和进入次数^[26]。因此, 结合本实验结果, 说明AD小鼠具有显著性焦虑, 而有氧运动可有改善焦虑样行为。

DAO主要存在于肠黏膜上皮细胞中, 当肠道黏膜受损时, DAO会被释放到血液中^[27]。而D-LA是肠道内细菌分解糖类后产生的一种代谢产物, 通常人体自身无法代谢D-LA, 因此其在血液中的水平可以反映肠道菌群的代谢状态以及肠道屏障功能^[28]。本实验发现AD小鼠血浆OAO、D-LA、LPS显著性上升, 有氧运动后显著性下降, 表明AD小鼠表现为肠黏膜

屏障功能下降, 肠道细菌代谢物过量进入血液, 而有氧运动可能对小鼠肠黏膜屏障具有保护作用。对结肠形态进行观察, 发现AD小鼠结肠上皮细胞受损, 柱状细胞较少且排列紊乱, 进一步证实了AD小鼠存在肠道屏障破坏。有氧运动使AD小鼠结肠黏膜上皮变厚, 柱状细胞增多且排列规则, 说明血浆OAO、D-LA、LPS的减少可能是由于运动调控肠道黏膜, 增强其屏障功能实现的。但近年来研究发现, 运动可调节AD肠道微生物丰度, 减少有害细菌, 增加益生菌^[29], 运动使AD小鼠血浆D-LA、LPS水平下降, 与其调控肠道微生物进而减少D-LA、LPS产生有关, 还是得益于屏障功能增强后的阻挡作用, 还有待进一步研究确认。

紧密连接是肠黏膜屏障的关键生理结构, 起到选择性渗透屏障的作用, 将身体与外部环境隔离开来, 保护身体免受微生物、毒素和抗原侵入到内环境^[30]。ZO-1、Occludin和Claudin-1是紧密连接屏障功能的关键组成部分, 是上皮细胞旁转运的决定因素^[31]。LIN等^[32]发现, 增加结肠炎小鼠紧密连接蛋白Claudin-1的表达水平, 导致肠道通透性降低和肠黏膜屏障功能障碍改善, 表明Claudin-1是维持肠道黏膜屏障的关键蛋白。FENG等^[33]发现, Occludin上调促进了肠黏膜屏障的形成并保护肠道屏障免受LPS损害, 这表明Occludin是肠黏膜执行屏障功能的关键成分。而体外和体内研究表明, ZO-1的下调与紧密连接通透性增加有关, KUO等^[34]证实ZO-1对于



A: 小鼠结肠TLR4蛋白相对表达量; B: 小鼠结肠MyD88蛋白相对表达量; C: 小鼠结肠NF- κ B蛋白相对表达量; D: 小鼠结肠TNF- α 蛋白相对表达量; E: 小鼠结肠IL-1 β 蛋白相对表达量; 数据以 $\bar{x} \pm s$ 表示, $n=5$, * $P<0.05$, ** $P<0.01$ 。

A: relative expression of TLR4 protein in mice colon; B: relative expression of MyD88 protein in mice colon; C: relative expression of NF- κ B protein in mice colon; D: relative expression of TNF- α protein in mice colon; E: relative expression of IL-1 β protein in mice colon; Data are expressed as $\bar{x} \pm s$, $n=5$, * $P<0.05$, ** $P<0.01$.

图6 小鼠结肠TLR4/MyD88/NF- κ B信号通路相关蛋白和炎症因子蛋白表达水平

Fig.6 Mouse colonic TLR4/MyD88/NF- κ B pathway and inflammatory factor protein expression levels

肠上皮增殖的上调和有丝分裂的成功完成至关重要,在肠黏膜修复中起着决定性作用。AD与肠道黏膜屏障功能有关,AD模型小鼠在肠上皮中表现出过多的A β 沉积,并伴有肠道通透性增加、炎症改变和紧密连接蛋白表达水平降低^[35]。WANG等^[36]发现,APP/PS1转基因AD小鼠肠道Occludin、Claudin-1、Claudin-5和ZO-1表达水平降低。最近的研究报道,有氧运动通过调节肠上皮紧密连接蛋白的表达来改善肠黏膜屏障功能。例如,GAO等^[37]发现,有氧运动通过提高ZO-1和Occludin的表达水平来恢复结肠和回肠之间的紧密连接,从而增强肠道屏障功能。SHIN等^[38]证明,有氧运动上调老年小鼠肠道ZO-1、

Occludin和Claudin-1表达,改善屏障功能。本研究结果表明,AD小鼠肠ZO-1、Occludin和Claudin-1表达水平降低,导致肠黏膜通透性增加和屏障功能受损,有氧运动显著增加了ZO-1、Occludin和Claudin-1的表达量,降低了肠黏膜屏障通透性,这与以前的发现一致。有研究提出,肠黏膜紧密连接完整性受Occludin和Claudin的磷酸化状态而改变,并可能受到所进行运动类型的调节^[39];运动导致的肠道缺氧可能增加低氧诱导因子1 α (hypoxia-inducible factor 1 α , HIF-1 α)的表达量,并调节肠道微生物群影响紧密连接蛋白的功能、黏液的合成/降解和抗氧化作用,进而增强肠道屏障功能^[40],但运动调节AD小鼠肠黏膜屏障功能的具体

机制还有待进一步深入研究。

衰老、慢性应激等作用下会导致机体肠道微生物群紊乱和肠道屏障通透性增加。来源于肠道细菌的LPS接触到肠道免疫细胞会触发TLR4受体,激活TLR4/MyD88/NF- κ B信号通路导致下游炎症因子的产生,进一步加剧肠道屏障通透性破坏和LPS进入内环境^[41]。DAI等^[42]发现,抑制结肠TLR4/MyD88/NF- κ B信号通路可降低血清中IL-1 β 、IL-6、TNF- α 和IL-18的含量,减轻溃疡性结肠炎,并采用TLR4敲除小鼠证实了TLR4的参与作用。本实验发现AD小鼠结肠TLR4通路蛋白和下游TNF- α 、IL-1 β 蛋白表达量显著性增加,而在有氧运动后显著性下降,表明有氧运动可抑制TLR4/MyD88/NF- κ B信号通路减少炎症因子的产生。但这是否与运动使LPS减少从而弱化了该通路的激活有关,还有待进一步证实。LPS过量进入血液后会形成内毒素血症,导致炎症因子释放和血脑屏障破坏,LPS进一步进入大脑中产生一系列反应,进而出现抑郁和焦虑等情绪^[43]。如SHENTU等^[44]发现,LPS诱导小鼠脑退化样家族成员4(vestigial like family member 4, VGLL4)、信号转导和转录激活因子3(signal transducer and activator of transcription 3, STAT3)、IL-1 β 上调,激活小胶质细胞,产生焦虑样行为。LI等^[45]发现,LPS诱导小鼠脑神经炎症和氧化应激,下调VGF神经生长因子诱导蛋白(VGF nerve growth factor inducible, VGF)和脑源性神经营养因子(brain-derived neurotrophic factor, BDNF),产生记忆缺陷和焦虑抑郁样行为。因此,本研究发现有氧运动可减轻AD小鼠焦虑样行为,可能与其保护肠道黏膜屏障,减少LPS位移有关,但运动具有全身效应,这些变化与大脑变化的因果关联还有待后续进一步研究确认。

综上所述,有氧运动可改善AD小鼠肠黏膜屏障功能,其作用机制与上调肠道紧密连接蛋白,弱化LPS位移,抑制TLR4/MyD88/NF- κ B信号通路减少炎症因子释放有关。运动可能通过调控AD小鼠肠黏膜功能减少LPS位移来改善焦虑样行为。本研究为有氧运动干预AD神经精神症状提供了证据,为AD的发病和防治提供了新的视角,但有氧运动改善AD焦虑样行为的大脑机制还有待进一步深入研究。

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