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大麻二酚及其纳米制剂对小鼠抑郁行为的作用

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摘要 目的 研究大麻二酚(CBD)及其纳米制剂对小鼠抑郁样行为的影响及机制。方法 腹腔注射脂多糖(LPS)诱导急性焦虑抑郁小鼠模型。将55只小鼠随机分组: 长期实验分为正常(Con)组、模型(LPS)组、大麻二酚(CBD)组、纳米大麻二酚(NCBD)组、舍曲林(SER)组, 每组7只; 短期实验分为Con组、LPS组、CBD组、NCBD组, 每组5只。除Con组和LPS组给予蒸馏水, 其余组灌胃给予25、50 mg/kg CBD及其纳米制剂。采用旷场和强迫游泳实验检测小鼠焦虑及抑郁样行为, Luxol Fast Blue 髓鞘染色检测前额皮层中髓鞘形态, 免疫荧光染色检测前额皮层中沉默信息调节因子2(SIRT2)、离子钙结合衔接分子1(Iba-1)和白细胞介素-1 β (IL-1 β)蛋白表达。结果 长期实验中与Con组比较, LPS组小鼠穿梭次数减少($P < 0.05$), 不动时间百分比增加($P < 0.01$), 髓鞘数量及长度减少($P < 0.05$); 与LPS组相比, CBD组、NCBD组、SER组小鼠抑郁样行为减少($P < 0.01$), 髓鞘数量及长度增加($P < 0.05$)。短期实验中与Con组比较, LPS组小鼠焦虑抑郁样行为增加($P < 0.05$), SIRT2表达降低($P < 0.01$), Iba-1、IL-1 β 表达增加($P < 0.01$); 与LPS组相比, CBD组、NCBD组小鼠焦虑样行为减少($P < 0.05$), SIRT2表达增加($P < 0.01$), Iba-1、IL-1 β 表达降低($P < 0.05$)。结论 CBD及其纳米制剂可缓解小鼠焦虑、抑郁样行为, 作用机制可能与逆转前额皮层中SIRT2蛋白表达、脱髓鞘变化、小胶质细胞活化及炎症因子的水平相关。

关键词 抑郁样行为; 大麻二酚; 沉默信息调节因子2; 白细胞介素-1 β ; 髓鞘; 纳米大麻二酚; 离子钙结合衔接分子1

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大麻二酚(cannabidiol, CBD)是一种植物大麻素成分, 无成瘾性^[1]。近年来, CBD作为一种潜在的抗焦虑药物引起了越来越多的关注^[2]。CBD作用靶点广泛, 可通过调节多种神经递质、受体、酶等发挥抗抑郁作用^[3]。沉默信息调节因子2(sirtuin2, SIRT2)是一种NAD⁺依赖性脱乙酰酶, 可以调节髓鞘形成、炎症等过程并与抑郁症相关^[4]。纳米药物制剂能极大程度上增强口服药物的吸收和生物利用度, 其研究和开发已成为当前医药学界热点^[5]。该研究利用脂多糖(lipopolysaccharide, LPS)诱导焦虑抑郁模型, 观察小鼠行为学表现, 检测小鼠前额皮层髓鞘形态及SIRT2、离子钙结合衔接分子1(ionized

calcium binding adapter molecule-1, Iba-1)、白细胞介素-1 β (interleukin-1 β , IL-1 β)蛋白表达水平, 初步探究CBD及其纳米制剂对LPS诱导小鼠抑郁样行为的缓解作用及相关机制。

1 材料与方法

1.1 材料

1.1.1 实验动物 SPF级ICR雄性小鼠55只, 体重18~22 g, 购自昆明医科大学动物部, 许可证号: SCXK(滇)K2020-0004。由昆明医科大学伦理审查委员会审批, 动物伦理审查批号: KM-MU20241867。小鼠常规适应性饲养1周后进行实验。

1.1.2 主要试剂和仪器 CBD(批号: GL20221222)购自西安格林生物科技有限公司; 纳米大麻二酚(nano-cannabidiol, NCBD)(批号: 20230101)购自湖州普瑞生物医药公司; 盐酸舍曲林片(sertraline hydrochloride, SER)(批号: FX3423), 国药准字H10980141, 购自大连辉瑞制药有限公司; LPS(货号: L2880D)购自北京博奥拓达公司; LFB染色试剂盒(货号: ab150675)购自英国Abcam公司; FITC二抗(货号: S0007)和SIRT2抗体(货号: AF5256)均购

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自美国 Affinity 公司; Iba-1 抗体(货号: GTX632426) 购自美国 GeneTex 公司; Cy3 二抗(货号: AS007) 购自武汉爱博泰克生物科技有限公司; 含 DAPI 的抗荧光衰减封片剂(货号: S2110) 购自北京索莱宝生物科技有限公司; IL-1 β 抗体(货号: bs-6319R) 购自北京博奥森生物技术有限公司。徕卡显微镜(型号: Leica DM4B) 购自德国徕卡公司; 荧光倒置显微镜(型号: Axio Observer, Z1) 购自德国卡尔·蔡司公司。

1.2 方法

1.2.1 小鼠抑郁模型的建立 小鼠适应性饲养 1 周后随机分组, 对照组腹腔注射生理盐水, 其余各组均采用 LPS 诱导抑郁模型。方法如下: LPS 用生理盐水稀释后进行腹腔注射(长期实验 2 mg/kg, 每日 1 次, 共 5 d; 短期实验 4 mg/kg, 每日 1 次, 共 3 d)。

1.2.2 分组及干预方式 分组及给药情况如下: 长期实验将小鼠随机分为 5 组: 对照(Con)组、模型(LPS)组、大麻二酚治疗(CBD)组、纳米大麻二酚治疗(NCBD)组、舍曲林(SER)组, 每组 7 只, Con 组和 LPS 组给予蒸馏水, SER 组灌胃舍曲林 10 mg/kg, CBD 组及 NCBD 组灌胃 CBD 及其纳米制剂 25 mg/kg, 连续 10 d。短期实验分为 4 组: Con 组、LPS 组、CBD 组、NCBD 组, 每组 5 只, CBD 组及 NCBD 组灌胃 CBD 及其纳米制剂 50 mg/kg, 单次给药。

1.2.3 小鼠的焦虑抑郁样行为的检测 参照文献^[9], 于最后 1 次注射 LPS 24 h 后进行旷场实验和强迫游泳实验。通过 SMART 3.0 软件记录小鼠 6 min 内的活动情况, 并分析小鼠的中央区域运动时间、路程百分比和穿梭次数。旷场实验结束 1 h 后进行强迫游泳实验, 观察 6 min, 分析小鼠后 4 min 内不动时间占比。通过这些行为学实验评定小鼠的焦虑抑郁程度。

1.2.4 Luxol fast blue 髓鞘染色检测小鼠前额皮层

中脱髓鞘程度 行为学实验结束后, 各组小鼠麻醉后灌注取脑, 进行石蜡切片(厚度 10 μ m)。切片在室温下与牢固蓝染液作用 20 h。蒸馏水冲洗后用碳酸锂溶液及 70% 乙醇分色, 与焦油紫溶液作用 5 min 后脱水透明, 用中性树胶封片并在显微镜下观察前额皮层中同区域各组小鼠脱髓鞘程度。

1.2.5 免疫荧光染色检测小鼠前额皮层中 SIRT2、Iba-1、IL-1 β 表达情况 石蜡切片(厚度 7 μ m) 经脱蜡脱二甲苯处理后在柠檬酸抗原修复液中热修复 15 min, 山羊血清室温封闭 2 h。加入一抗(比例参照说明书) 4 $^{\circ}$ C 孵育过夜, PBS 洗涤后加入 Cy3 二抗或 FITC 二抗, 室温避光孵育 2 h。PBS 洗涤后, 滴加含 DAPI 的抗荧光衰减封片剂封片后在显微镜下观察, 采集前额皮层同一区域染色结果图, 使用 Image J 软件分析平均荧光强度。

1.3 统计学处理 采用 SPSS 19.0 软件处理数据, GraphPad Prism 9 作图。结果以 $\bar{x} \pm s$ 表示, 多组间总体比较用单因素方差分析, 两两组间比较用 LSD 检验, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 CBD 及其纳米制剂对小鼠焦虑抑郁样行为的影响 长期实验结果显示, LPS 组较 Con 组小鼠旷场实验穿梭次数减少($P < 0.05$), 强迫游泳不动时间百分比增加($P < 0.01$); CBD 组、NCBD 组和 SER 组较 LPS 组小鼠不动时间百分比降低(均 $P < 0.01$), 见表 1。短期实验中 LPS 组较 Con 组小鼠穿梭次数、中央运动时间百分比以及中央运动路程百分比减少($P < 0.05$), 强迫游泳不动时间百分比增加($P < 0.05$); CBD 组及 NCBD 组较 LPS 组穿梭次数、中央运动时间百分比增加(均 $P < 0.01$), 见表 2。这些数据表明 CBD 及其纳米制剂可以缓解 LPS 诱导小鼠的焦虑抑郁样行为。

表 1 长期实验中 5 组小鼠旷场实验和强迫游泳实验中行为的比较($n = 7, \bar{x} \pm s$)

Tab. 1 Comparison of behaviors of five groups of mice in open field experiment and forced swimming experiment in long-term experiment ($n = 7, \bar{x} \pm s$)

Group	Time in central area (%)	Distance in central area (%)	Zone transition number	Immobility time in forced swimming experiment (%)
Con	10.27 $\pm$ 2.75	17.47 $\pm$ 2.40	50.56 $\pm$ 8.24	0.04 $\pm$ 0.01
LPS	5.32 $\pm$ 1.00	12.84 $\pm$ 3.05	28.71 $\pm$ 5.39*	0.15 $\pm$ 0.03**
CBD	8.37 $\pm$ 2.02	15.78 $\pm$ 2.41	45.29 $\pm$ 8.68	0.07 $\pm$ 0.02##
NCBD	14.37 $\pm$ 2.33 [#]	21.03 $\pm$ 2.53 [#]	43.57 $\pm$ 5.88	0.05 $\pm$ 0.01 ^{##}
SER	16.00 $\pm$ 5.13 [#]	21.18 $\pm$ 3.36 [#]	53.86 $\pm$ 8.63 [#]	0.03 $\pm$ 0.01 ^{##}
F value	2.139	1.636	1.683	9.708
P value	0.100	0.191	0.180	<0.001

* $P < 0.05$, ** $P < 0.01$ vs Con group; [#] $P < 0.05$, ^{##} $P < 0.01$ vs LPS group.

2.2 CBD 及其纳米制剂对 LPS 小鼠前额皮层髓鞘的影响 髓鞘染色结果显示,与 Con 组小鼠相比,LPS 组小鼠前额皮层髓鞘数量及长度减少 ($P < 0.05$); 与 LPS 组小鼠相比,CBD 组 ($P < 0.01$)、

NCBD 组 ($P < 0.05$)、SER 组 ($P < 0.01$) 髓鞘数量增加,表明 CBD 及其纳米制剂长期给药可改善 LPS 诱导小鼠的脱髓鞘。见图 1。

2.3 CBD 及其纳米制剂对 LPS 小鼠前额皮层中

表 2 短期实验中 4 组小鼠旷场实验和强迫游泳实验中的行为的比较 ($n = 5, \bar{x} \pm s$)

Tab. 2 Comparison of behaviors of four groups of mice in open field experiment and forced swimming experiment in short-term experiment ($n = 5, \bar{x} \pm s$)

Group	Time in central area (%)	Distance in central area (%)	Zone transition number	Immobility time in forced swimming experiment (%)
Con	6.70 ± 1.41	14.38 ± 1.50	36.60 ± 5.98	0.02 ± 0.01
LPS	$1.83 \pm 0.41^*$	$7.47 \pm 1.56^*$	$11.60 \pm 2.64^*$	$0.10 \pm 0.03^*$
CBD	$9.30 \pm 1.25^{##}$	$15.15 \pm 2.78^{\#}$	$46.60 \pm 7.18^{##}$	0.06 ± 0.06
NCBD	$10.76 \pm 1.87^{##}$	$18.76 \pm 1.41^{##}$	$43.40 \pm 9.22^{##}$	0.19 ± 0.11
<i>F</i> value	8.537	6.173	5.612	6.115
<i>P</i> value	0.001	0.005	0.008	0.006

* $P < 0.05$ vs Con group; $^{\#}P < 0.05$, $^{##}P < 0.01$ vs LPS group.

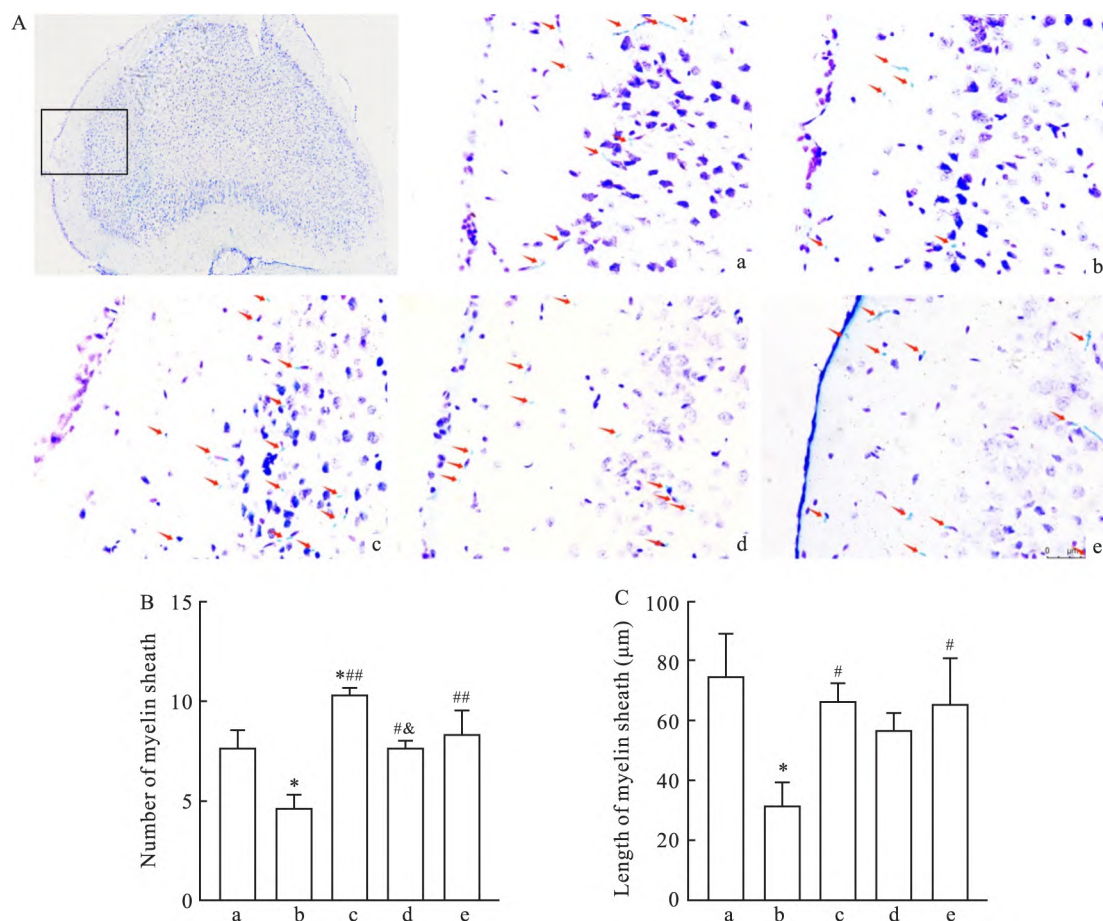


图 1 LFB 髓鞘染色法检测 CBD 及其纳米制剂对小鼠前额皮层中髓鞘的影响

Fig. 1 The effects of CBD and its nano-preparation on myelin sheath in mouse prefrontal cortex detected by LFB myelin sheath staining method

A: Light microscope picture of LFB myelin sheath staining $\times 400$; Red triangle arrow: myelin sheath; B: Comparison of myelin sheath number in each group; C: Comparison of myelin sheath length in each group; a: Con group; b: LPS group; c: CBD group; d: NCBD group; e: SER group; * $P < 0.05$ vs Con group; $^{\#}P < 0.05$, $^{##}P < 0.01$ vs LPS group; $^{\&}P < 0.05$ vs CBD group.

SIRT2、Iba-1、IL-1 β 蛋白表达的影响 免疫荧光实验结果显示(图 2A、C、E),与 Con 组相比,LPS 组小鼠前额皮层区域 SIRT2 表达下降($P < 0.05$); Iba-1 水平、IL-1 β 水平显著增加(均 $P < 0.01$),该区域可能存在小胶质细胞及炎症反应过度激活。CBD 组

及 NCBD 组较 LPS 组小鼠 SIRT2 表达增加($P < 0.01$),Iba-1、IL-1 β 表达下降($P < 0.05$),提示 CBD 及其纳米制剂在 50 mg/kg 剂量下可增加 LPS 小鼠前额皮层中的 SIRT2 蛋白表达并抑制小胶质细胞活化及炎症反应。见图 2B、D、F。

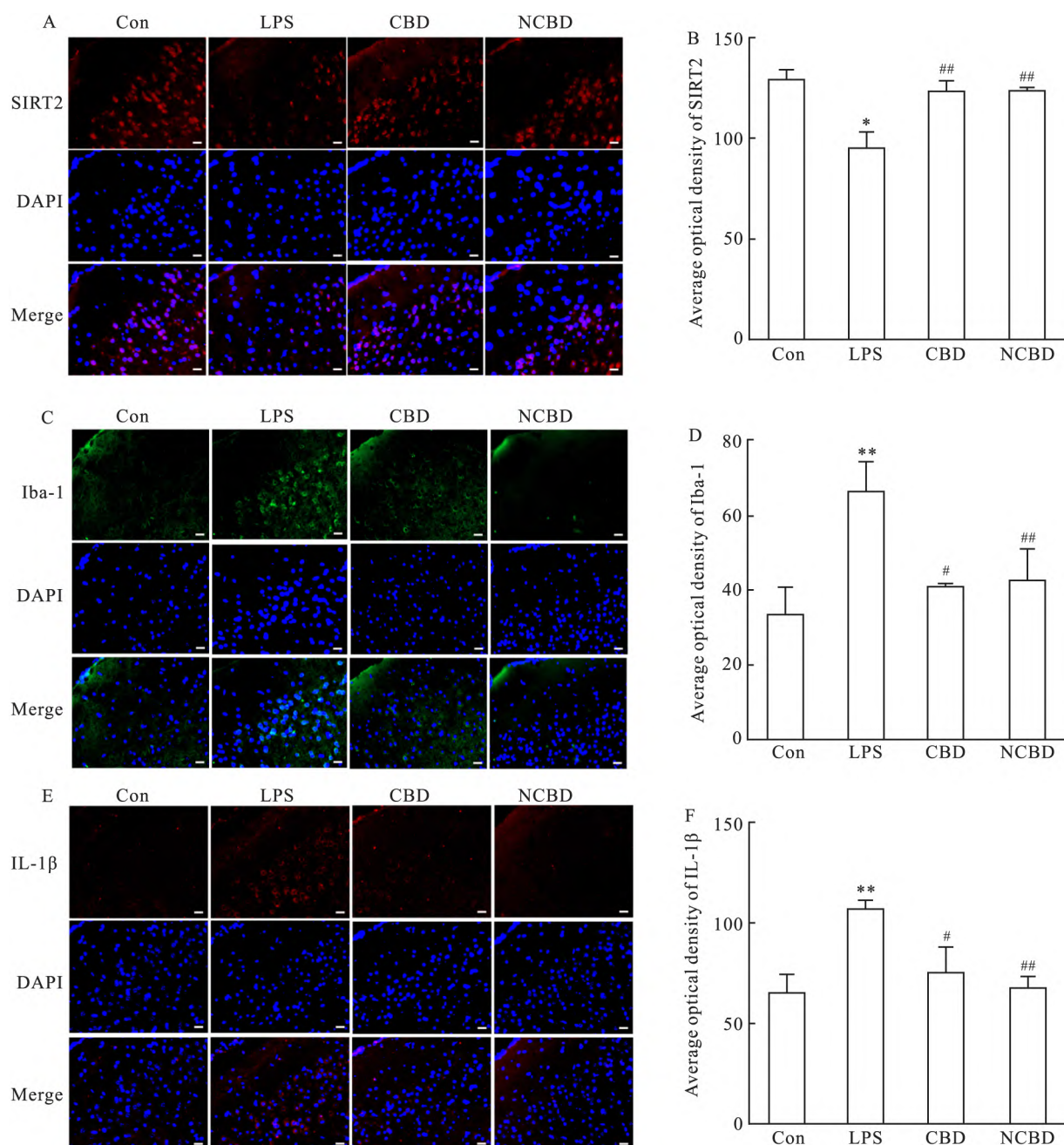


图 2 免疫荧光染色检测各组小鼠前额皮层中 SIRT2、Iba-1、IL-1 β 蛋白表达情况

Fig. 2 The expression of SIRT2, Iba-1 and IL-1 β protein in the prefrontal cortex of mice in each group detected by immunofluorescence staining

A: SIRT2 immunofluorescence picture $\times 400$; B: Comparison of average fluorescence intensity of SIRT2 in each group; C: Iba-1 immunofluorescence picture $\times 400$; D: Comparison of the average fluorescence intensity of Iba-1 in each group; E: IL-1 β immunofluorescence picture $\times 400$; F: Comparison of average fluorescence intensity of IL-1 β in each group; * $P < 0.05$, ** $P < 0.01$ vs Con group; # $P < 0.05$, ## $P < 0.01$ vs LPS group.

3 讨论

抑郁症是多种因素共同作用的结果。过大的心理压力会过度刺激免疫系统,导致促炎作用与抗炎作用的失衡,促炎因子的表达增加会促进抑郁样行为的发展^[7]。LPS 作为一种炎症诱导剂,可激活单核细胞、巨噬细胞,导致炎症因子水平升高。这些外周炎症信号通过内皮细胞到达中枢神经系统,引发神经炎症,加重抑郁症状^[8]。CBD 是一种无毒、不具精神活性且不良反应少的植物大麻素成分,具有抗焦虑、抗感染和抗抑郁等多种药理作用。CBD 的抗焦虑和抗抑郁特性与剂量和时间过程(急性与慢性)密切相关^[9]。临床研究^[10]表明 CBD 在急性给药时可以治疗广泛性焦虑症、社交焦虑症,而有关其急性给药治疗抑郁症及慢性给药疗效的研究较少。课题组前期研究^[11]表明,采用低剂量(25 mg/kg)长期给药可以改善慢性束缚应激小鼠的焦虑抑郁样行为并上调前额皮层及海马中 BDNF、突触素等因子的表达,而采用 50 mg/kg 剂量长期给药对慢性束缚应激小鼠的焦虑抑郁样行为无影响,但可以上调海马区域突触素、雷帕霉素靶蛋白表达。基于该研究结果,本文采用低剂量(25 mg/kg)长期给药以及高剂量(50 mg/kg)急性单次给药探索 CBD 及其纳米制剂对 LPS 小鼠焦虑抑郁样行为的影响及相关机制。

长期给药行为学实验结果显示,LPS 诱导小鼠的强迫游泳不动时间百分比增加,CBD 及其纳米制剂给药后降低,提示 CBD 及其纳米制剂长期给药(25 mg/kg)可以改善 LPS 诱导小鼠的抑郁样行为。短期行为学实验结果显示,LPS 诱导小鼠的旷场实验中央运动时间、路程百分比减少,强迫游泳不动时间百分比增加,CBD 及其纳米制剂给药后增加了中央运动时间、路程百分比,但对强迫游泳不动时间无明显影响,这表明 CBD 及其纳米制剂单次给药(50 mg/kg)能缓解 LPS 小鼠焦虑样行为,但对抑郁样行为无缓解作用。但由于本次行为学实验也存在动物例数偏少这一不足,需扩大例数以获得更科学更全面的结论。

抑郁小鼠前额皮层区域出现抑郁相关蛋白表达异常^[12]。髓鞘是一层管状结构,紧密包裹在有鞘神经纤维的轴突周围,其可以加速神经冲动的传导。在抑郁小鼠模型中,观察到髓鞘的长度和厚度有所减少^[13]。本研究结果显示,LPS 诱导小鼠前额皮层出现脱髓鞘,CBD 及其纳米制剂长期给药(25 mg/

kg) 后髓鞘数量及长度增加,表明 CBD 及其纳米制剂可以缓解 LPS 小鼠前额皮层的脱髓鞘。小胶质细胞是中枢神经系统的固有免疫细胞,当脑内发生炎症或感染时,小胶质细胞迅速响应,激活其吞噬能力。这种激活状态的小胶质细胞作为神经炎症的标志物,其在炎症过程中的活性变化是导致抑郁情绪产生的重要机制之一^[14]。SIRT2 是去乙酰化酶类 Sirtuins 家族的一员,参与调控神经炎症反应并与抑郁行为相关。LPS 刺激小鼠后可导致脑组织内 SIRT2 蛋白表达水平降低,小胶质细胞被激活及炎症因子 TNF- α 、IL-1 β 水平增加,用 SIRT2 抑制剂处理后小胶质细胞被进一步激活且 TNF- α 、IL-1 β 水平进一步增加^[15]。

本研究结果显示,CBD 及其纳米制剂给药后较模型组小鼠前额皮层中 SIRT2 表达增加,Iba-1 及 IL-1 β 表达降低,提示 CBD 及其纳米制剂抗焦虑、抑郁作用可能与逆转前额皮层中 SIRT2 蛋白水平、脱髓鞘变化、小胶质细胞的活化及炎症因子的表达相关。

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The effect of cannabidiol and its nano-preparation on depressive behaviors in mice

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Abstract Objective To investigate the therapeutic effects and underlying mechanisms of cannabidiol (CBD) and its nano-formulations on depression-like behaviors in mice. **Methods** A murine model of acute anxiety and depression was established by intraperitoneal administration of lipopolysaccharide (LPS). A total of 55 mice were randomly assigned into several groups: for the long-term study, a control group (Con), a model group (LPS), a cannabidiol group (CBD), a nano-cannabidiol group (NCBD), and a sertraline (SER) group, each consisting of 7 mice. In the short-term study, mice were divided into four groups: the Con group, LPS group, CBD group, and NCBD group, with 5 mice in each group. Except for the Con group and LPS group, which were given distilled water, the remaining groups were administered 25 and 50 mg/kg of cannabidiol and its nano-formulation via oral gavage. The open field and forced swimming tests were employed to assess anxiety- and depression-like behaviors in

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immune cells. The expression of Card9 in the cornea of mice treated with Card9 siRNA was significantly reduced. After inhibiting the expression of Card9 gene, the expressions of Card9, NF- κ B, IL-1 β , IL-6 and TNF- α significantly decreased and the changes of IL-1 β were most significant. In *in vitro* studies, Card9 exhibited negligible expression in human corneal epithelial cells, contrasting with its pronounced expression in THP-1 cells. After the induction of macrophages, Card9, NF- κ B, IL-1 β , IL-6, and TNF- α were significantly upregulated under the stimulation of *A. fumigatus*. After inhibiting the expression of Card9, the stimulated expression of these factors was significantly reduced, with the most notable change observed in IL-1 β . **Conclusion** Card9 is involved in the inflammatory development and healing process of *A. fumigatus* keratitis. Card9 deficiency can cause functional impairment of macrophages and inhibit the expression of inflammatory factors to a certain extent, in which IL-1 β has the greatest effect.

Key words Card9; *Aspergillus fumigatus*; macrophage; inflammatory factor; keratitis

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mice. Luxol Fast Blue myelin staining was employed to evaluate myelin sheath morphology in the prefrontal cortex, and immunofluorescence staining was utilized to quantify the protein expression levels of silencing information regulator (SIRT2), ionized calcium binding adaptor molecule-1 (Iba-1), and interleukin-1 β (IL-1 β) in the prefrontal cortex. **Results** In the long-term experiment, the LPS group exhibited a significant reduction in shuttle times ($P < 0.05$), an increase in immobility time ($P < 0.01$), and a decrease in the number and length of myelin sheaths ($P < 0.05$) compared to the Con group. Compared to the LPS group, the depressive behaviors in the CBD, NCBD, and SER groups were significantly alleviated ($P < 0.01$), and the number and length of myelin sheaths increased ($P < 0.05$). In the short-term experiment, compared to the Con group, the LPS group exhibited significantly increased anxiety- and depression-like behaviors ($P < 0.05$), downregulated SIRT2 expression ($P < 0.01$), and upregulated Iba-1 and IL-1 β expression ($P < 0.01$). The CBD and NCBD groups demonstrated a reduction in anxiety and depression-like behaviors ($P < 0.05$), an increase in SIRT2 expression ($P < 0.01$), and a decrease in Iba-1 and IL-1 β expressions ($P < 0.05$) compared to the LPS group. **Conclusion** CBD and its nano-formulations effectively mitigate anxiety and depression-like behaviors in mice. The underlying mechanisms may be associated with the reversal of SIRT2 protein expression, demyelination changes, microglial activation, and the levels of inflammatory factors in the prefrontal cortex.

Key words depression-like behaviors; cannabidiol; silent information regulator 2; interleukin-1 β ; myelin sheath; nano-cannabidiol; ionized calcium binding adapter molecule-1

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