论著·神经科学

硼化镁纳米颗粒通过上调BDNF减轻海马神经元及突触损伤改善糖尿病并发抑郁症小鼠行为和脑结构异常

左晓飞[1],范华菊[2],吴倩盈[1],殷 昊[1],陈琪椿[1],张华鑫[1],潘文青[3],王英帅[1],杨娜娜[1] 山东第二医科大学:[1]生命科学与技术学院,[3]基础医学院,山东潍坊;[2]四川省第二退伍军人医院心理健康服务中心,四川成都

[摘要] **目的** 糖尿病并发抑郁症(diabetes mellitus complicated with depression,DD)是糖尿病患者常见的精神心理共病,表现为持续情绪低落以及糖代谢紊乱加重,严重影响疾病预后与患者生存质量。本研究通过构建DD小鼠模型,探讨硼化镁(MgB_2)纳米颗粒对其行为和脑结构的影响。**方法** 本研究利用扫描电子显微镜、X射线衍射对所制备的MgB_2纳米颗粒进行表征,利用氢气微电极检测该纳米颗粒在体外以及体内胃、肠组织和血液中的释氢性能,通过动物实验评估其体内安全性。40只7~8周龄雄性C57BL/6J小鼠(体质量18~23 g)按照随机数字表法分为对照(Control)组、模型(DD)组、20 mg/kg MgB_2组、30 mg/kg MgB_2组和40 mg/kg MgB_2组。DD组和3个不同剂量的MgB_2组小鼠通过一次性腹腔注射链脲佐菌素(streptozotocin,STZ,150 mg/kg),在第5 d测空腹血糖(fasting blood glucose,FBG),若FBG≥11.3 mmol/L则持续喂养7 d,随后连续7 d腹腔注射脂多糖(lipopolysaccharide,LPS,0.5 mg/kg)来构建DD小鼠模型,Control组小鼠注射等剂量的柠檬酸-柠檬酸钠缓冲溶液。DD造模成功后,MgB_2组小鼠连续灌胃对应剂量的MgB_2纳米颗粒治疗7 d,其余组小鼠灌胃等体积的生理盐水。末次灌胃治疗24 h后通过行为学实验确定最佳治疗剂量。最佳MgB_2纳米颗粒剂量治疗后检测小鼠体质量、血清葡萄糖(glucose,GLU)含量;脑部MRI观察小鼠海马体体积、各向异性分数(fractional anisotropy,FA)和平均弥散系数(mean diffusivity,MD);ELISA检测小鼠海马脑源性神经营养因子(brain-derived neurotrophic factor,BDNF);透射电子显微镜观察海马超微结构的变化。**结果** MgB_2纳米颗粒在酸性条件下具有优异释氢特性,且释放过程均匀稳定。该材料兼具高储氢效能和安全性,可在胃内快速释放,在肠组织中缓慢释放,在血液中维持稳定。在旷场实验中,3个不同剂量的MgB_2组小鼠在中央区域和外周区域的运动距离和穿格次数较DD组显著增加($P<0.01$);在高架十字迷宫实验中,3个不同剂量的MgB_2组小鼠在开放臂上的停留时间与运动距离较DD组显著增加($P<0.05$),在封闭臂上的停留时间显著降低、运动距离较DD组显著增加($P<0.01$);在悬尾实验和强迫游泳实验中,3个不同剂量的MgB_2组小鼠的不动时间较DD组显著缩短($P<0.01$)。以上行为学实验结果发现3种剂量的MgB_2纳米颗粒组间疗效相当,因此选择20 mg/kg作为MgB_2纳米颗粒的最佳治疗剂量。与DD组相比,MgB_2纳米颗粒能够有效增加小鼠的体质量[(21.85±1.24) *vs* (19.30±1.20)g,$P<0.01$]、海马体积[(3.77±0.68) *vs* (2.63±0.47)mm^3,$P<0.01$]、BDNF[(2 295.78±259.47) *vs* (1 913.69±262.05)pg/mL,$P<0.05$]和FA(左侧:0.23±0.03 *vs* 0.16±0.02;右侧:0.22±0.04 *vs* 0.15±0.02,$P<0.01$),降低血清GLU含量[(25.97±4.66) *vs* (38.25±8.42)mmol/L,$P<0.01$]和MD[左侧:(5.11±0.09)×10^{-4} *vs* (5.42±0.23)×10^{-4} mm^2/s;右侧:(5.19±0.45)×10^{-4}

[基金项目] 国家自然科学基金青年科学基金项目(81600360);山东省自然科学基金重点项目(ZR2020KH008);山东省自然科学基金创新发展联合基金项目(ZR2024LSW011);鸢都学者课题(000007060);江苏悟空生物科技有限公司横向项目(02191004)

[通信作者] 杨娜娜,E-mail:benben1980@126.com
王英帅,E-mail:yingshuaiwang1987@163.com

2097-0927/Copyright © 2026,陆军军医大学(第三军医大学)。这篇开放获取文章遵循CC BY许可协议(https://creativecommons.org/licenses/by/4.0/)

引用格式:左晓飞,范华菊,吴倩盈,等. 硼化镁纳米颗粒通过上调BDNF减轻海马神经元及突触损伤改善糖尿病并发抑郁症小鼠行为和脑结构异常[J]. 陆军军医大学学报,2026,48(17):2440-2452. DOI:10.16016/j.2097-0927.202604004.

Citation:Zuo X F,Fan H J,Wu Q Y,*et al*. Magnesium diboride nanoparticles alleviate hippocampal neurons and synapses by upregulating BDNF to improve behavioral and brain structural abnormalities in diabetes mellitus mice complicated with depression[J]. J Army Med Univ,2026,48(17):2440-2452. DOI:10.16016/j.2097-0927.202604004.

vs (5.60±0.27)×10^{-4} mm^2/s,*P*<0.05],使得海马神经元核膜完整、核内染色质较均匀,细胞器较丰富,海马突触数量增加(5.33±1.22 *vs* 3.56±1.50,*P*<0.05),突触间隙增加[(21.66±2.72) *vs* (17.23±2.69)nm,*P*<0.01],结构较为清晰。**结论** MgB_2纳米颗粒通过上调BDNF水平,减少对海马神经元和突触的损伤,从而改善DD小鼠的行为和脑结构异常。

[**关键词**] MgB_2;糖尿病并发抑郁症;海马;脑源性神经营养因子
[**中图法分类号**] R587.2;R749.4;R914.3 [**文献标志码**] A

Magnesium diboride nanoparticles alleviate hippocampal neurons and synapses by upregulating BDNF to improve behavioral and brain structural abnormalities in diabetes mellitus mice complicated with depression

Zuo Xiaofei[1], Fan Huaju[2], Wu Qianying[1], Yin Hao[1], Chen Qichun[1], Zhang Huaxin[1], Pan Wenqing[3], Wang Yingshuai[1], Yang Nana[1] [1]School of Life Science and Technology; [3]School of Basic Medicine, Shandong Second Medical University, Weifang, Shandong; [2]Mental Health Service Center, Sichuan Provincial Second Veterans Hospital, Chengdu, Sichuan, China

[**Abstract**] **Objective** Diabetes mellitus complicated with depression (DD) is a common psychiatric comorbidity in diabetic patients, characterized by persistent low mood and disordered glucose metabolism, which severely impacts disease prognosis and patient's quality of life. This study aims to investigate the effects of magnesium diboride (MgB_2) nanoparticles on the behavior and brain structure of mice in a mouse model of DD. **Methods** Scanning electron microscopy and X-ray diffraction were performed to characterize the prepared MgB_2 nanoparticles, and hydrogen microelectrodes were employed to detect the hydrogen release properties of the nanoparticles *in vitro* and in the gastric, intestinal, and blood tissues in mice. *In vivo* safety was evaluated through animal experiments. Forty male C57BL/6J mice (7 to 8 weeks old, weighing 18 to 23 g) were randomly divided into a Control group, a model (DD) group, and 20, 30 and 40 mg/kg MgB_2 groups. The mice in the DD group and the 3 MgB_2 groups were administered a single intraperitoneal injection of streptozotocin (STZ, 150 mg/kg), and fasting blood glucose (FBG) was measured on day 5. If FBG was ≥11.3 mmol/L, the mice were fed continuously for 7 d, followed by consecutive intraperitoneal injections of lipopolysaccharide (LPS, 0.5 mg/kg) for 7 d to establish the DD mouse model, while the mice in the Control group were injected with an equal dose of citric acid-sodium citrate buffer. After successful DD modeling, the mice in the MgB_2 groups were intragastrically administered corresponding doses of MgB_2 nanoparticles for 7 consecutive days, while the mice in the other groups were given an equivalent volume of normal saline by gavage. Behavioral tests were performed 24 h after the final administration to determine the optimal therapeutic dose. Following treatment with the optimal dose of MgB_2 nanoparticles, body weight and serum glucose (GLU) levels were measured. Brain MRI scanning was performed to assess hippocampal volume, fractional anisotropy (FA) and mean diffusivity (MD). Hippocampal brain-derived neurotrophic factor (BDNF) levels were measured by ELISA. Changes in hippocampal ultrastructure were observed by transmission electron microscopy. **Results** MgB_2 nanoparticles exhibited excellent hydrogen release properties under acidic conditions, with a uniform and stable release process. The nanoparticles possessed both high hydrogen storage capacity and safety, enabling rapid hydrogen release in the stomach, slow release in intestinal tissue, and stable maintenance in the blood. In the open field test, the mice in the 3 MgB_2 groups showed significant increases in the traveled distance and the crossing numbers in both the central and peripheral zones compared with the DD group (*P*<0.01). In the elevated plus maze test, treatment of MgB_2 nanoparticles resulted in increased time spent and traveled distance on the open arms compared with those in the DD group (*P*<0.05), decreased time spent and more traveled distance in the closed arms compared with that in the DD group (*P*<0.01). In the tail suspension and forced swim tests, the immobility time of mice in the 3 different MgB_2 dose groups was significantly shorter than that of the DD group (*P*<0.01). These behavioral findings indicated comparable efficacy among the 3 MgB_2 nanoparticle doses; consequently, 20 mg/kg was selected as the optimal therapeutic

dose. Compared with the DD group, MgB_2 nanoparticles effectively increased body weight [(21.85±1.24) *vs* (19.3±1.20) g, *P*<0.01] hippocampal volume [(3.77±0.68) *vs* (2.63±0.47) mm^3, *P*<0.01], BDNF expression [(2 295.78±259.47) *vs* (1 913.69±262.05) pg/mL, *P*<0.05], and FA (Left side: 0.23±0.03 *vs* 0.16±0.02; right side: 0.22±0.04 *vs* 0.15±0.02, *P*<0.01), while reducing serum GLU levels [(25.97±4.66) *vs* (38.25±8.42) mmol/L, *P*<0.01], and MD [Left side: (5.11±0.09)×10^{-4} *vs* (5.42±0.23)×10^{-4} mm^2/s; right side: (5.19±0.45)×10^{-4} *vs* (5.60±0.27)×10^{-4} mm^2/s, *P*<0.05]. These improvements resulted in intact nuclear membranes in hippocampal neurons, relatively uniform intranuclear chromatin, abundant organelles, increased number of hippocampal synapses (5.33±1.22 *vs* 3.56±1.50, *P*<0.05), elevated synaptic cleft width [(21.66±2.72) *vs* (17.23±2.69) nm, *P*<0.01], and clearer structural features. **Conclusion** MgB_2 nanoparticles improve behavioral and structural abnormalities in the brains of DD mice by upregulating BDNF levels and reducing damage to hippocampal neurons and synapses.

[**Key words**] MgB_2; diabetes mellitus complicated with depression; hippocampus; brain-derived neurotrophic factor

Supported by the National Natural Science Foundation for Young Scholars of China (81600360), the Key Project of Natural Science Foundation of Shandong Province (ZR2020KH008), the Joint Innovation and Development Project of Natural Science Foundation of Shandong Province (ZR2024LSW011), the Yuandu Scholar Foundation (000007060), and the Horizontal Project Funded by Jiangsu Wukong Biotechnology Co., LTD (02191004). Corresponding authors: Yang Nana, E-mail: benben1980@126.com; Wang Yingshuai, E-mail: yingshuaiwang1987@163.com

糖尿病(diabetes mellitus,DM)属于一种慢性代谢性疾病,核心病理表现为血糖水平持续性升高。全球范围内DM患病人数在2024年已经突破5亿,预计到2050年将接近9亿[1]。抑郁症作为DM中最常见的精神心理并发症之一,二者间存在明显病理关联。近年来,糖尿病并发抑郁症(diabetes mellitus complicated with depression,DD)的患病率存在明显的升高趋势。对DM患者中抑郁症的患病率进行Meta分析发现,在DM患者中,DD的患病率高达35%[2]。DD所致疾病负担在世界范围内持续加重,已成为一种全球性健康挑战。

海马体作为大脑中参与情绪调节与认知功能的核心脑区[3],在DD中出现明显的结构和功能障碍。动物研究表明,DD小鼠的海马区域会出现明显的神经损伤,而给予诺卡酮治疗后可减轻海马神经元损伤,从而改善小鼠的抑郁样行为[4]。此外,神经影像学方面证实,DM患者和抑郁症患者的海马区域会出现严重萎缩[5-6]。脑源性神经营养因子(brain-derived neurotrophic factor,BDNF)作为中枢神经系统中分布最广泛的一类神经营养因子,参与神经、免疫和代谢调控,对中枢神经系统功能稳态及机体代谢平衡具有关键病理生理学意义[7]。临床研究表明,DD患者的BDNF水平较正常人群显著降低,而增加BDNF的表达有助于控制血糖、改善抑郁症状[8]。进一步证实了BDNF在DD海马功能障碍中的关键作用。

目前,DD的治疗主要依赖药物治疗、心理干预等方式治疗,但这些方法普遍存在价格昂贵、不良反应多、治疗效果有限等问题[9-10],因此开发安全、耐受性良好的治疗策略成为当前研究的重点方向。氢气(H_2)作为一种理化性质稳定的简单气体分子,在抗氧化、抗炎、抗凋亡等方面展现出多重生物学活性。已有研究证实H_2可显著改善糖代谢异常,缓解急性和慢性应激所诱发的焦虑和抑郁样行为,并对DM模型大鼠海马区结构损伤及神经元凋亡产生抑制效应[11-13],H_2已成为当前医学研究中最具有潜力的生物活性气体之一。然而,H_2的有效递送是制约其广泛应用的关键问题之一。本研究采用硼化镁(MgB_2)纳米颗粒作为氢递送载体,经检测其产氢的同时还兼具安全性和高储氢效能。在此基础上通过构建DD小鼠模型,旨在探讨该纳米颗粒对DD小鼠行为和脑结构的影响。

1 材料与方法

1.1 实验动物

本研究选用86只7~8周龄SPF级雄性C57BL/6J小鼠,体质量18~23 g,由山东第二医科大学医学实验动物中心提供。所有小鼠均饲养于山东第二医科大学医学实验动物中心SPF级动物房,环境温度维持在(22±2)℃,相对湿度45%~55%,光照周期设定为12 h/12 h明暗交替,实验期间小鼠食物与饮水供应保持充足且无任何限制。实验全程采取最大措施减轻动物疼痛与应激损伤:动物抓取、药物注射、手术操作均配合规范麻醉镇痛;每日定时观察动物的精神状态,进食饮水情况。当动物体质量减轻到原体质量的20%~25%、持续不能进食饮水或濒死状态等人道终点时,小鼠吸入浓度1.5%的异氟烷麻醉后脱颈处死。所有程序均遵循山东第二医科大学实验动物正确护理和使用指

南,并经山东第二医科大学实验动物护理和使用委员会批准(2023SDL242)。

1.2 试剂与仪器

链脲佐菌素(streptozotocin,STZ)购自山东思科捷生物技术有限公司;脂多糖(lipopolysaccharide,LPS)购自美国Sigma公司;柠檬酸钠和柠檬酸均购自中国阿拉丁试剂(上海)有限公司;MgB_2颗粒购自上海巷田纳米材料有限公司;葡萄糖(glucose,GLU)试剂盒和尿素氮(blood urea nitrogen,BUN)测试盒购自中国南京建成生物工程研究所;丙氨酸氨基转移酶(alanine aminotransferase,ALT)测试试剂盒购自深圳迈瑞生物医疗电子股份有限公司;BDNF试剂盒购自苏州正鑫医药有限公司。氢气微电极购自丹麦UNISENSE公司;高架十字迷宫、旷场箱、悬尾测试箱、强迫游泳桶、小动物吸入式麻醉机均购自中国瑞沃德生命科技有限公司;酶标仪购自美国Thermo Fisher Scientific公司;透射电子显微镜(transmission electron microscope,TEM)和扫描电子显微镜(scanning electron microscope,SEM)购自日本Hitachi公司;7.0 T小动物活体成像系统购自德国Bruker公司。

1.3 方法

1.3.1 MgB_2纳米颗粒的制备与表征 将适量MgB_2颗粒置于玛瑙研钵中研磨10 min,随后转移至离心管中,并加入等质量无水乙醇,重悬后4 000 r/min离心取中层在室温下真空干燥,并密封保存,即得到MgB_2纳米颗粒。采用SEM检测MgB_2纳米颗粒的形貌与粒径;使用X射线衍射(X-ray diffraction,XRD)分析其物相,以综合表征其微观结构特征。

1.3.2 检测MgB_2纳米颗粒在不同pH下的释氢性能 为探究MgB_2纳米颗粒在不同pH环境中释氢性能,本研究精确称取3 mg MgB_2纳米颗粒,置于不同pH值(1.2、6.0、7.4)的15 mL生理盐水体系中,通过轻柔搅拌促进反应进行,将氢气微电极检测器浸入反应体系,连续在线检测24 h内氢释放情况。体系内产氢速率由仪器自动连续采集,为消除仪器采样噪声、数据波动带来的误差,所有原始数据均进行平滑降噪处理。累计产氢浓度(Q)通过对产氢速率与时间进行积分获得:

$$Q = \int_{t_1}^{t_2} v_{H_2}(t)dt$$

式中,Q为累计产氢浓度(μmol/L);$v_{H_2}(t)$为t时刻对应产氢速率;t_1和t_2积分区间设定反应起始时间和反应终止时间(h)。本研究中,积分区间为0~24 h,基线设为0。

由于本研究检测数据为大量离散采样点,依托Origin 9软件采用梯形数值积分法完成上述定积分的数值求解,离散数据计算公式:

$$Q \approx \sum_{i=1}^{n-1} \frac{v_{i+1} + v_i}{2} \cdot (t_{i+1} - t_i)$$

式中,n代表采样点总数;v_i和t_i分别代表第i个采样点对应产氢速率与采样时间;$t_{i+1} - t_i$为相邻2组间采样点的时间间隔。本研究共收集到8 638个采样点。

1.3.3 MgB_2纳米颗粒的安全性检测 将16只C57BL/6J小鼠按随机数字表法分为MgB_2纳米颗粒组与Control组,每组8只:MgB_2纳米颗粒组小鼠连续7 d灌胃大剂量(40 mg/kg)的MgB_2纳米颗粒;Control组小鼠灌胃相同体积的生理盐水。在末次灌胃24 h后,检测小鼠血清中的ALT和BUN水平,HE染色观察小鼠的心脏、肝脏、脾脏、肺、肾脏以及海马组织的病理变化。

1.3.4 检测MgB_2纳米颗粒在胃、肠组织和血液中的释氢性能 将30只正常雄性C57BL/6J小鼠按随机数字表法分为5组,每组6只,使用MgB_2纳米颗粒(20 mg/kg)灌胃,分别在灌胃后1、3、6、12和24 h(n=6)内使用氢气微电极检测H_2浓度。小鼠吸入1.5%异氟烷麻醉后,沿腹中线切开腹腔,插入氢气微电极测定胃、肠组织中H_2浓度;摘眼球取血,将血液滴入肝素管中,稀释5倍后即刻插入氢气微电极进行测定血液中H_2浓度。

1.3.5 DD模型的构建 C57BL/6J小鼠适应性饲养1周后,禁食12 h,按照本课题组前期的方法来构建DD小鼠模型[14]:小鼠通过一次性腹腔注射STZ(150 mg/kg),注射5 d后禁食1晚,使用采血针从尾端静脉取血测空腹血糖(fasting blood glucose,FBG),若FBG≥11.3 mmol/L,则正常饲养7 d(若FBG<11.3 mmol/L,小鼠禁食12 h后补充注射1次STZ,剂量100 mg/kg),随后连续7 d每日腹腔注射1次LPS(0.5 mg/kg)来构建DD小鼠模型。Control组小鼠注射等剂量的柠檬酸-柠檬酸钠缓冲溶液。

1.3.6 不同MgB_2纳米颗粒治疗DD小鼠的最佳剂量筛选 将40只C57BL/6J小鼠按随机数字表法分为Control组、DD组和20 mg/kg MgB_2组、30 mg/kg MgB_2组和40 mg/kg MgB_2组,每组8只。MgB_2组在DD模型构建成功后,将MgB_2纳米颗粒分散于生理盐水,纳米颗粒剂量分别为20 mg/kg(2 mg/mL)、30 mg/kg(3 mg/mL)和40 mg/kg(4 mg/mL),每日灌胃1次,持续7 d;Control组与DD组灌胃等体积的生理盐水。末次灌胃24 h后,进行行为学实验以评估确定最佳治疗剂量。

1.3.6.1 旷场实验(open field test,OFT)评估小鼠焦虑水平 末次灌胃治疗24 h后进行OFT实验。本研究采用40 cm×40 cm×40 cm的塑料露天场地[15],小鼠经过1 min的适应后,采用智能行为分析系统(Smart 3.0)

与海康威视摄像设备连续记录5 min内小鼠在中央区域和外周区域的运动距离及穿格次数,并对其相关行为进行评估。

1.3.6.2 高架十字迷宫实验(elevated plus maze test,EPM)评估小鼠焦虑样行为 EPM作为评估实验动物焦虑样行为的常用行为学方法[15],该装置是由黑色塑料板制成,包含2个开放臂(30 cm×5 cm×15 cm)、2个相同大小的封闭臂和1个中央区域(5 cm×5 cm)。该装置距离地面60 cm。实验时,将小鼠置于十字迷宫中央,使用智能行为分析系统(Smart 3.0)与海康威视摄像设备记录小鼠5 min内在开放臂和封闭臂的停留时间和运动距离。

1.3.6.3 悬尾实验(tail suspension test,TST)评估小鼠抑郁样行为 使用透明胶带将小鼠尾部单独悬挂在箱体,小鼠适应2 min后,使用智能行为分析系统(Smart 3.0)与海康威视摄像设备记录4 min内的不动时间。

1.3.6.4 强迫游泳实验(forced swim test,FST)评估小鼠抑郁样行为 将小鼠置于透明塑料圆筒(高30 cm,直径18 cm),筒内注水深度约18 cm,水温维持在(24±1)℃。小鼠适应2 min后,采用智能行为分析系统(Smart 3.0)与海康威视摄像设备记录4 min内的不动时间[16]。

1.3.7 脑部磁共振成像(magnetic resonance imaging,MRI)扫描 末次灌胃24 h后,小鼠吸入1.5%异氟烷麻醉,将其头部固定于扫描床线圈内。成像序列及参数设置如下,①T2加权成像(T2-weighted imaging,T2WI):重复时间(repetition time,TR)3 000 ms,回波时间(echo time,TE)34 ms,视野(field of view,FOV)31 mm×31 mm,层厚0.5 mm,层数30层,矩阵160×150;②弥散张量成像(diffusion tensor imaging,DTI):TR 3 500 ms,TE 26 ms,FOV 25 mm×25 mm,层厚0.5 mm,层数30层,矩阵128×128。

1.3.8 血清和海马组织样本采集 脑部MRI扫描完成24 h内,小鼠吸入1.5%异氟烷麻醉处理后实施采血,所获血液经离心处理以分离血清。血样采集完毕后,用颈椎脱臼方式处死小鼠,将小鼠完整脑组织取出后,用镊子小心剥离出所需要的海马,随后转移至-80 ℃低温冰箱中保存,以便后续各项指标的测定。

1.3.9 小鼠体质量和血清GLU含量检测 行为学和MRI扫描结束后对小鼠进行称重。将分离好的血清从-80 ℃冷冻环境中取出,置于室温缓慢解冻,血清GLU含量测定严格遵循试剂盒操作规程执行。

1.3.10 ELISA检测海马组织中的BDNF表达水平 将小鼠海马组织进行准确称量,按照质量(g):体积(mL)=1:9比例加入生理盐水,冰水浴条件下研磨组织制成匀浆,3 000 r/min离心10 min后收集上清液。ELISA检测步骤参考试剂盒说明执行。

1.3.11 海马超微结构检测 将小鼠海马组织切至1.0 mm×1.0 mm×1.0 mm大小后,立即浸入3%戊二醛内固定1~2 h。固定后用预冷的PBS在4 ℃条件下漂洗3次,随后加入1%锇酸,4 ℃条件下固定2 h后用PBS漂洗3次,每次10 min。洗涤后依次以50%、70%、90%乙醇梯度脱水。样本经无水丙酮与包埋剂按3:1、1:1、1:3及纯包埋剂比例完成浸透后,转入包埋模板,注入包埋剂后,温箱聚合设定为37 ℃ 12 h、45 ℃ 12 h以及60 ℃ 24 h。聚合完成后进行修块、定位及超薄切片,切片厚度为70 nm,并将切片收集到铜网上。依次用醋酸双氧铀染色及枸橼酸铅进行染色,干燥后,用HITACHIHT 7700观察。

1.4 统计学分析

所有实验数据采用SPSS 27.0版本软件进行处理分析,并用GraphPad Prism 8.0和Origin 9软件绘制统计图表。数据以$\bar{x}\pm s$表示,多组间比较采用单因素方差分析,两组间比较采用独立样本t检验分析,$P<0.05$表示差异具有统计学意义。

2 结果

2.1 MgB_2纳米颗粒表征以及在不同pH条件下释氢性能

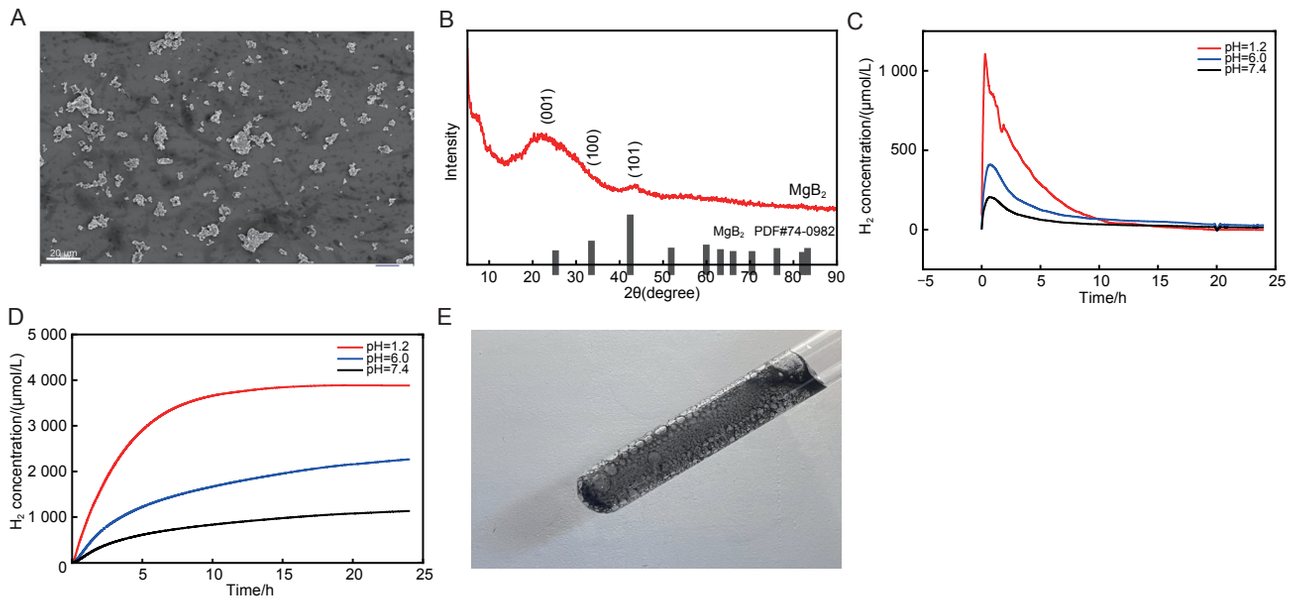
本实验所制备的MgB_2纳米颗粒平均粒径为750 nm(图1A)。XRD结果表明,该材料仍保持六方MgB_2的晶体结构,未见明显杂质相生成(图1B)。为评估其释氢性能,本研究在体外模拟不同pH值(1.2、6.0、7.4)实验结果表明,该颗粒在酸性环境中产氢性能高于弱碱性环境,且酸性越强,H_2释放量越大且释放过程稳定(图1C、D)。此外,MgB_2纳米颗粒与生理盐水反应还可观察到明显气体生成现象(图1E)。

2.2 灌胃MgB_2纳米颗粒未引起小鼠主要脏器病理及肝肾功能指标的异常变化

HE染色结果显示,小鼠的心脏、肝脏、脾脏、肺、肾脏和海马组织并没有出现明显的病理变化(图2A)。MgB_2纳米颗粒组小鼠血清中的ALT和BUN水平与Control组小鼠血清中的水平相似(图2B、C),表明MgB_2纳米颗粒对小鼠的毒性极低,具有良好的安全性。

2.3 MgB_2纳米颗粒在胃内快速释放H_2,在肠组织中缓慢释放以及在血液中维持稳定

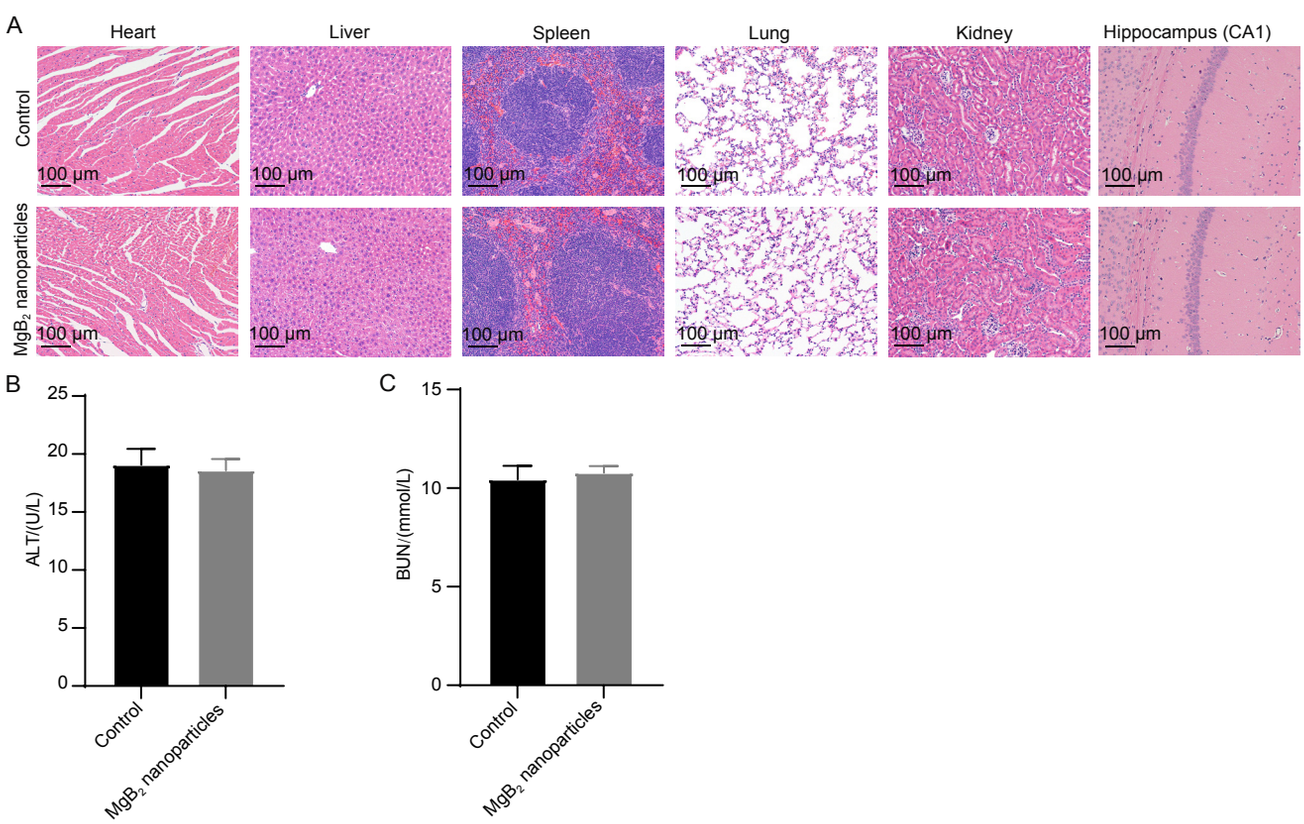
结果显示,MgB_2纳米颗粒(20 mg/kg)在胃中反应迅速,能快速且持续12 h产生H_2,而在肠组织中会缓慢产生24 h H_2。在血液中也持续检测到H_2的存在,但H_2浓度保持相对稳定(图3)。



A: Representative images of MgB_2 nanoparticles by scanning electron microscopy; B: XRD pattern of MgB_2 nanoparticles; C and D: H_2 production rate (C) and cumulative H_2 concentration (D) over 24 h at pH=1.2, 6.0 and 7.4; E: Photos of gas evolution from MgB_2 nanoparticles reacting with normal saline

图 1 MgB_2纳米颗粒的结构特性及不同pH条件下的释氢能力

Figure 1 Structural characteristics of MgB_2 nanoparticles and their hydrogen release capacity under different pH conditions



A: Representative images by HE staining showing the histological changes in the heart, liver, spleens, lung, kidneys, and hippocampus of mice in the control and MgB_2 nanoparticle groups; B and C: Serum ALT (B) and BUN (C) levels in the control and MgB_2 nanoparticle groups of mice following intragastric gavage

图 2 MgB_2纳米颗粒的安全性检测(n=8)

Figure 2 Safety testing of MgB_2 nanoparticles(n=8)

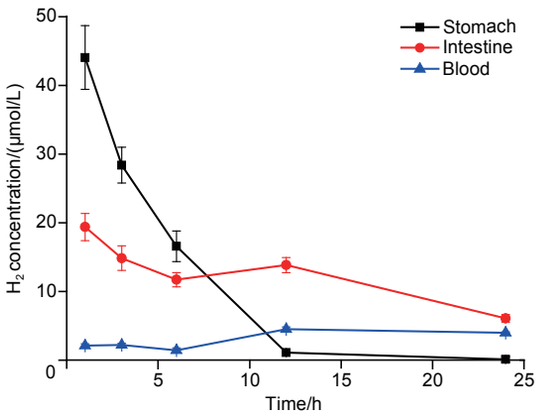
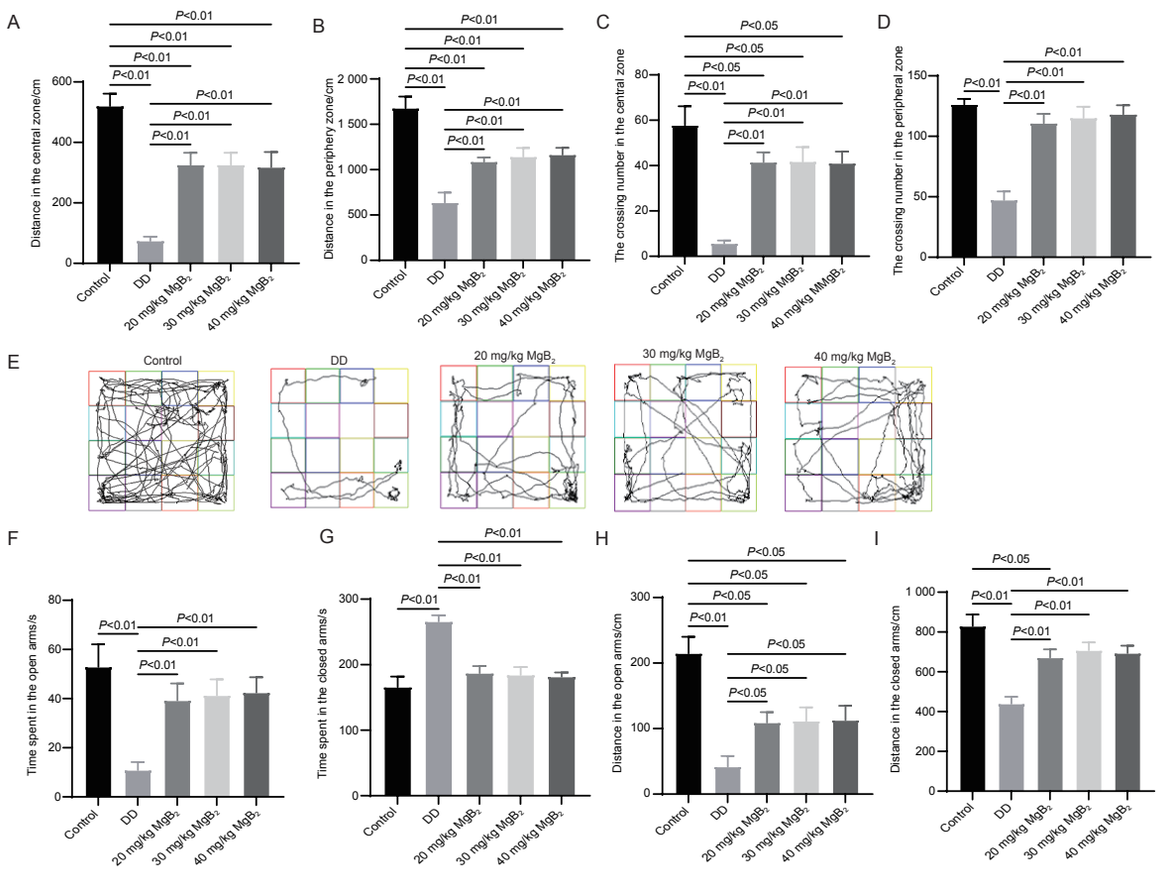


图3 MgB_2纳米颗粒在小鼠胃、肠组织和血液中的释氢性能(n=6)

Figure 3 Hydrogen-releasing properties of MgB_2 nanoparticles in the stomach, intestinal tissues and blood of mice(n=6)

2.4 MgB_2纳米颗粒改善DD小鼠焦虑和抑郁样行为

行为学实验结果表明,与Control组相比,DD组小鼠出现明显焦虑和抑郁样行为。经过MgB_2纳米颗粒治疗后,在OFT中,小鼠在中央区域与外周区域的运动距离和穿格次数与DD组相比均显著增加(P<0.01,图4A~E)。在EPM中,小鼠在开放臂的停留时间和运动距离与DD组相比均显著增加(P<0.05),同时,在封闭臂的停留时间均显著降低,在封闭臂的运动距离均显著增加(P<0.01,图4F~J)。在TST和FST中,小鼠不动时间与DD组相比均显著降低(P<0.01,图4K、L)。这3种剂量的MgB_2纳米颗粒组间疗效相当,基于最小有效剂量原则,考虑到安全性,本研究将20 mg/kg的MgB_2纳米颗粒作为最佳剂量。因此后续实验均采用20mg/kg的MgB_2纳米颗粒进行治疗。

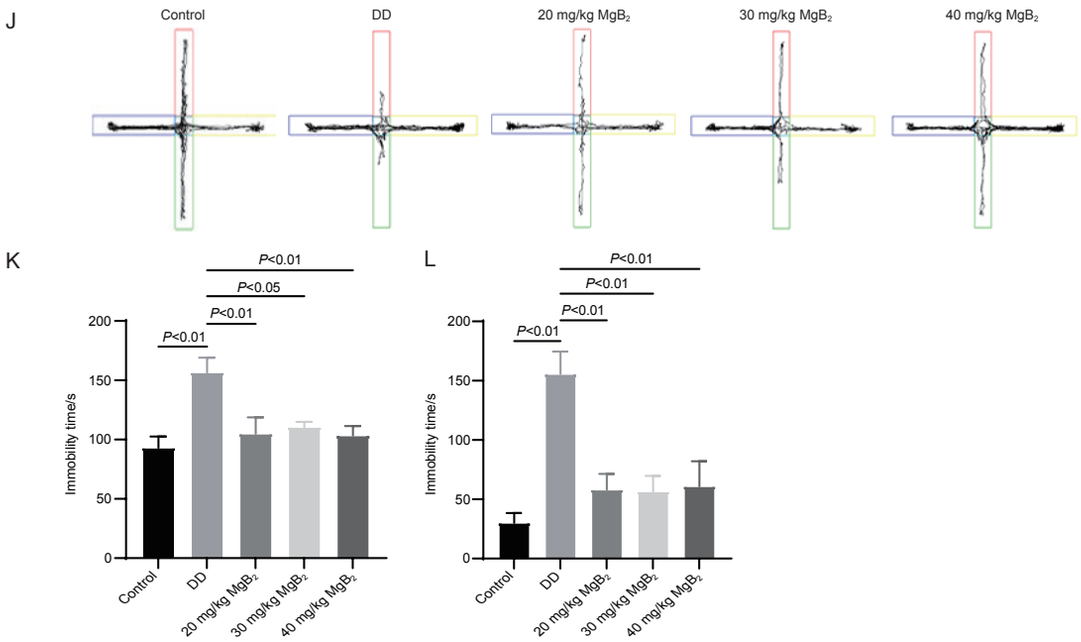


A and B: Traveled distance in the central (A) and peripheral (B) zones of the open field test among 3 dose groups of MgB_2 nanoparticles; C and D: The crossing numbers in the central (C) and peripheral (D) zones of the open field test among 3 dose groups of MgB_2 nanoparticles; E: Movement trajectory in the open field test; F and G: Time spent in the open (F) and closed (G) arms of the elevated plus maze test among 3 dose groups of MgB_2 nanoparticles; H and I: Traveled distance in the open (H) and closed (I) arms of the elevated plus maze test among 3 dose groups of MgB_2 nanoparticles

图4 行为学实验检测小鼠焦虑和抑郁样行为(n=8)

Figure 4 Behavioral tests to assess anxiety-like and depression-like behaviors in mice(n=8)

(图4 J~L续下页)



J: Movement trajectory in the elevated plus maze test; K and L: Immobility time in the tail suspension (K) and forced swim (L) tests among 3 dose groups of MgB_2 nanoparticles

续图4 行为学实验检测小鼠焦虑和抑郁样行为(n=8)

Figure 4(continued) Behavioral tests to assess anxiety-like and depression-like behaviors in mice(n=8)

2.5 MgB_2纳米颗粒可增加DD小鼠海马体体积并逆转DTI信号异常

DTI序列扫描显示,与Control组对比,DD组小鼠的海马体中的各向异性分数(fractional anisotropy,FA)显著降低(P<0.01),平均弥散系数(mean diffusivity,MD)显著增加(P<0.01),而MgB_2纳米颗粒(20 mg/kg)治疗后能有效地逆转FA和MD的异常变化(图5A~D)。T2WI成像显示,DD组小鼠的海马体积显著减小(P<0.01),MgB_2纳米颗粒(20 mg/kg)治疗后,海马体积显著增加(图5E、F)。

2.6 MgB_2纳米颗粒可抑制DD小鼠体质量和血清GLU含量的异常变化

结果显示,与Control组相比,DD组小鼠的体质量显著降低(P<0.01,图6A),血清GLU含量显著增加(P<0.01,图6B)。与DD组相比,MgB_2纳米颗粒(20 mg/kg)治疗后小鼠的体质量明显上升(P<0.01)、血清GLU含量明显降低(P<0.01)。

2.7 MgB_2纳米颗粒可上调DD小鼠海马组织的BDNF表达水平

结果显示,DD组小鼠相较于Control组小鼠海马组织的BDNF表达显著降低(P<0.01),而MgB_2纳米颗粒(20 mg/kg)治疗后显著上调了小鼠海马组织的BDNF表达(P<0.05),见图7。

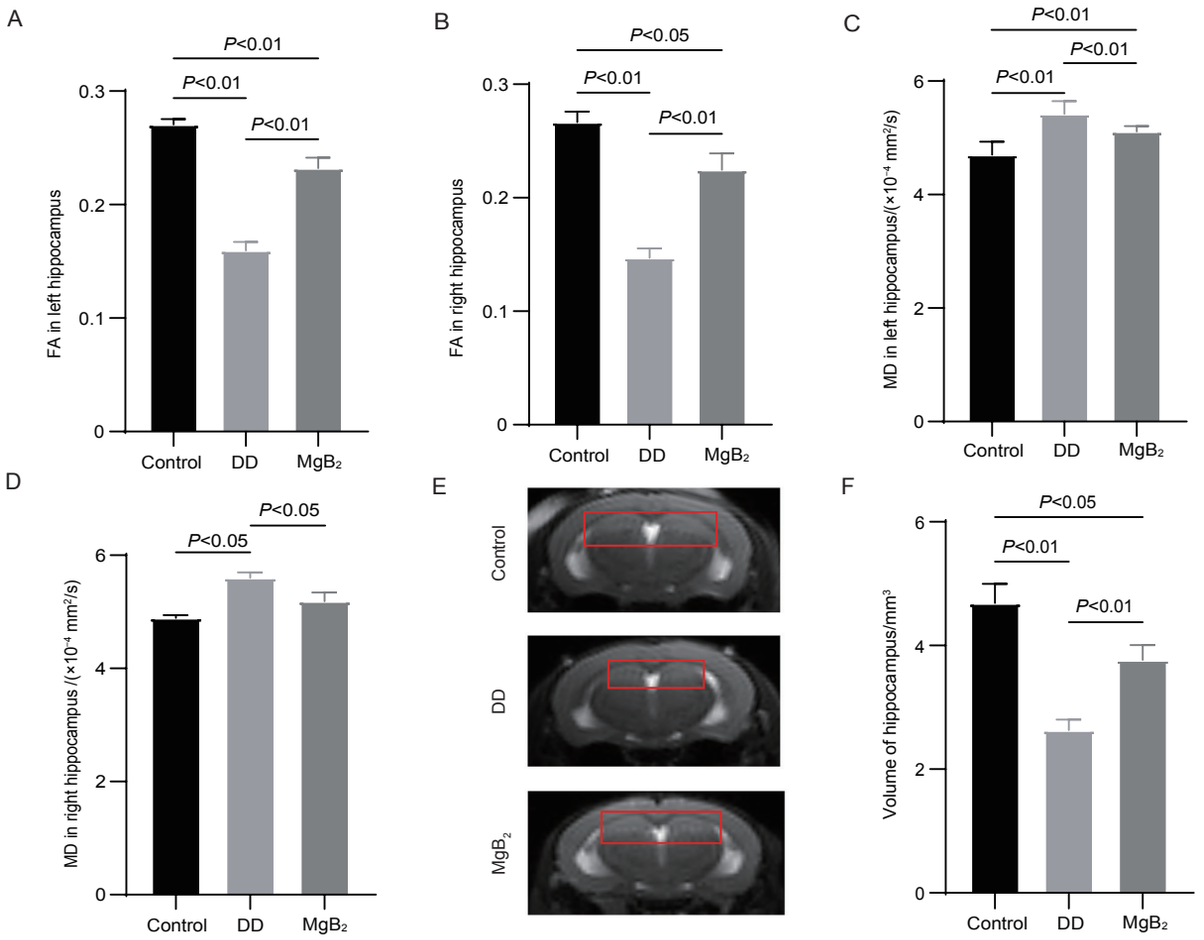
2.8 MgB_2纳米颗粒可改善DD小鼠海马神经元和突触结构损伤

TEM结果显示,Control组小鼠海马神经元结构正常,胞核边界清晰、核膜完整、核仁明显,染色质分布均匀,突触结构清晰完整,数量较多,囊泡分布均匀且含量丰富。与Control组相比,DD组小鼠海马神经元出现核膜破裂、核皱缩、核质散在,线粒体嵴结构被破坏的现象;与DD组相比,MgB_2组小鼠海马神经元核膜完整,细胞核形态趋于正常,核内染色质较均匀,细胞器较丰富,线粒体结构较完整(图8A)。与Control组相比,DD组小鼠海马突触结构松散,界限模糊不清,突触小泡融合或破裂(图8B);与DD组小鼠相比,MgB_2组小鼠海马突触数量增多(P<0.05)、突触间隙增加(P<0.01),结构较为清晰(图8C、D)。

3 讨论

3.1 炎症是连接DM与抑郁症的关键病理桥梁:STZ联合LPS构建DD模型的理论基础

DD是DM患者常见的慢性并发症之一,可显著增加患者自杀风险并加重疾病负担[17],由于其发病涉及多因素致病机制和系统间的复杂相互作用,单一动物模型难以完整再现其临床特征。以往DD的造模方式主要采用连续14 d灌胃高脂乳剂后,尾静脉一次性注



A and B: Quantitative fractional anisotropy (FA) analysis of the left hippocampus (A) and right hippocampus (B); C and D: Quantitative mean diffusivity (MD). analysis of the left hippocampus (C) and right hippocampus (D); E: T2WI of the hippocampus (the red box delineates the hippocampal region); F: Quantitative analysis of total bilateral hippocampal volume

图 5 MRI检测小鼠海马体的体积和DTI信号(n=8)

Figure 5 Hippocampal volume and DTI parameters measured by MRI(n=8)

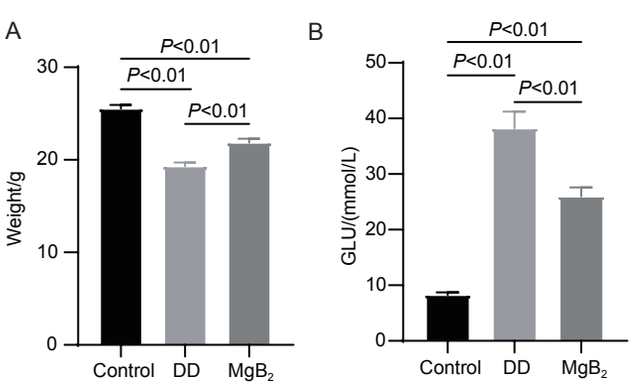


图 6 小鼠体质量(A)及血清GLU(B)含量检测(n=8)

Figure 6 Measurement of body weight (A) and serum GLU levels (B) in mice(n=8)

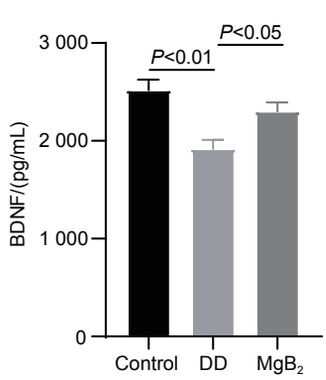
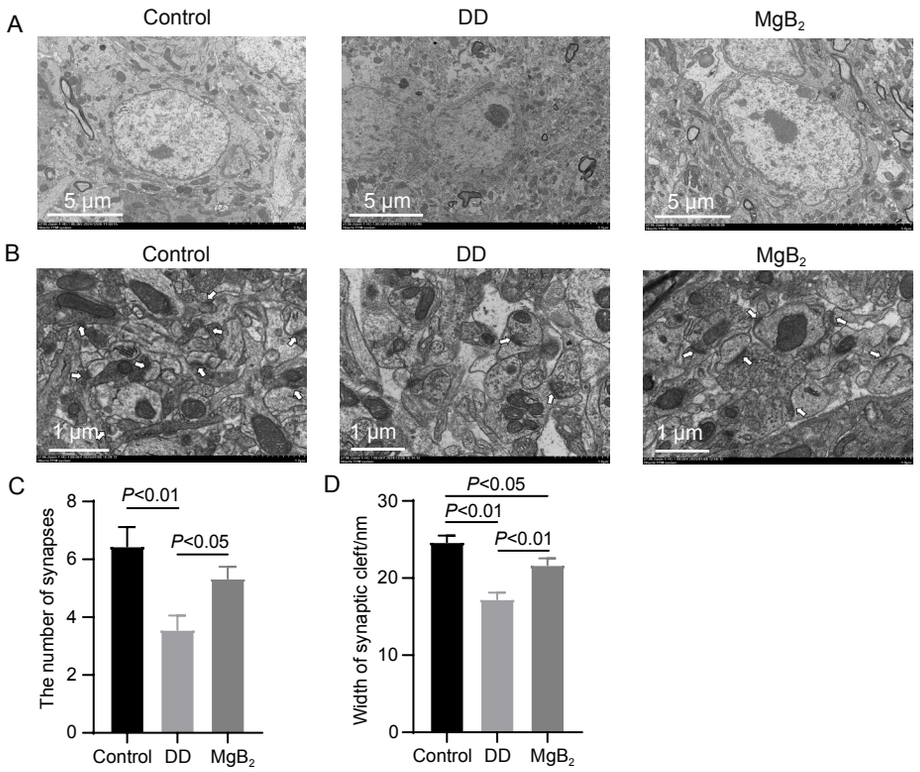


图 7 ELISA检测小鼠海马组织BDNF的含量(n=8)

Figure 7 BDNF levels in mouse hippocampal tissue by ELISA(n=8)



A: Hippocampal neuronal ultrastructure observed under TEM; B: Hippocampal synapse ultrastructure observed under TEM. White arrows indicate hippocampal synapses; C and D: Quantitative analyses of synapse number (C) and synaptic cleft width (D) in mice (n=3)

图 8 TEM观察小鼠海马神经元细胞和突触超微结构

Figure 8 Representative images of the ultrastructure of neurons and synapses in the mouse hippocampus by transmission electron microscopy (TEM)

射STZ和连续进行28 d慢性不可预测应激[18]或通过高脂饮食联合皮质酮[19]构建。其中,STZ作为经典DM诱导剂可诱导DM小鼠模型[20]。此外,LPS作为一种亲脂性分子,可穿过血脑屏障进入大脑,诱发神经炎症反应,从而引发小鼠认知障碍[21],原因在于其能激活小胶质细胞、促进炎症因子增加,并最终损伤海马神经可塑性[22-23]。值得注意的是,DM本身就伴有慢性炎症,且临床研究已证实DM患者与抑郁症患者均存在炎症水平升高的现象[24-25]。由此推测,炎症是连接DM与抑郁症共病的重要病理环节,因此本课题组通过联合腹腔注射STZ(150 mg/kg)和LPS(0.5 mg/kg)构建DD模型,该模型能够模拟人类DM患者因炎症诱导DD的发展过程。

3.2 长效释氢策略突破传统给氢局限:MgB_2纳米颗粒具有治疗DD的优势

H_2因其具有易获得、经济、无副作用等优点,表现出抗炎、抗氧化、抗凋亡功能,广泛应用于医疗领域。当前,常见的H_2摄入方式主要包括吸入法[26]和饮用富氢水[27]。虽然这些方式操作简单,但仍存在明显的局限性,如H_2吸入法需持续吸入且治疗场景受限,而饮用富氢水则存在疗效有限、持续时间短等问题。因此,开发能够长效维持的新型给氢策略,对提升H_2治疗的临床转化价值具有重要意义。本研究制备的MgB_2纳米颗粒在酸性条件下具有优良的释氢特性且释放过程良好,其疗效在抑郁行为改善、血糖控制以及改善海马损伤等水平上与课题组前期研究氢氧(H_2/O_2:66.7%/33.3%)混合气体吸入治疗DD的效果一致[14],提示无论是氢氧(H_2/O_2:66.7%/33.3%)混合气体吸入还是MgB_2纳米颗粒的给氢途径,均对DD产生相似的治疗效果,这为氢分子医学应用提供了新型递送策略。

3.3 上调BDNF表达可能是MgB_2纳米颗粒改善DD小鼠抑郁样行为的关键机制

海马是情绪调节的开关,在认知与情绪记忆之间发挥转换作用[28]。BDNF作为维持海马结构和功能的核心神经营养因子,在DD病理进程中发挥关键保护作用,这一点已得到临床和动物实验的双重验证。临床研究证实,DD患者血清BDNF水平显著低于非DD患者[29],动物实验亦表明,DD模型大鼠海马BDNF水

平较对照组同样显著降低[30]。更有研究指出,LPS诱导的神经炎症可破坏前额叶皮层、海马和伏隔核中的BDNF-TrkB信号传导,进而引发抑郁样表型[31],而藏红花油可通过外源性方式激活BDNF从而改善小鼠抑郁样行为[32]。表明海马BDNF信号受损是DD发生发展的关键环节,激活BDNF可作为DD干预的重要策略。

3.4 持续性H_2释放通过恢复海马体积及脑白质结构完整性来缓解DD小鼠神经损伤

在DD中,除海马BDNF的水平发生变化外,还伴有显著的脑部结构异常。MRI技术为DD的神经病理机制研究提供了无创的评估手段,该技术在肿瘤分子影像、脑功能成像等诸多领域的应用价值日益凸显。T2WI通过检测脑组织的横向弛豫时间差异从而显示脑部结构以及异常情况[33]。海马体萎缩与抑郁症、阿尔茨海默病等多种疾病的认知功能损伤密切相关[34]。临床表明DM患者[35]和抑郁症患者[6]均存在海马萎缩现象。DM患者海马萎缩源于慢性高血糖诱导的微血管病变、血脑屏障损伤和中枢胰岛素抵抗等,导致海马灌注不足和神经元代谢障碍[36-37]。抑郁症患者海马的变化与下丘脑-垂体-肾上腺轴的激活、糖皮质激素神经毒性及神经发生抑制密切相关[38]。而DTI是通过测量水分子弥散方向和弥散程度来反映白质微结构完整性[39],常用指标包括FA和MD[40]。本研究表明,MgB_2纳米颗粒(20 mg/kg)治疗后能够逆转海马体体积减小、FA显著降低和MD显著增加的现象。此外,TEM观察到MgB_2纳米颗粒改善DD小鼠海马神经元、突触损伤程度。

3.5 MgB_2纳米颗粒的改进策略

尽管MgB_2纳米颗粒可上调BDNF表达,逆转海马体积减小及减轻海马损伤来改善DD小鼠的行为和脑结构异常。但是,其性能仍需进一步优化,未来可通过石墨烯或聚合物包覆材料,提高MgB_2纳米颗粒的生物相容性和分散稳定性,同时构建表面壳层,防止其在生理环境中过早降解,从而保证H_2的可控释放。

综上所述,本研究制备的MgB_2纳米颗粒具有优良的产氢性能和良好的生物安全性。在DD小鼠模型中,MgB_2纳米颗粒可有效改善小鼠焦虑和抑郁样行为,该效应可能与上调海马BDNF表达,逆转海马萎缩及DTI信号异常有关,进而减少对海马神经元和突触的损伤,改善DD小鼠的行为和脑结构异常。

利益冲突 所有资助方仅为本研究提供资金支持,除上述资助外,所有作者与资助方均无其他利益关系,亦不存在其他利益冲突

参考文献:

[1] Duncan B B, Magliano D J, Boyko E J. IDF Diabetes Atlas 11th edition 2025: global prevalence and projections for 2050 [J]. Nephrol Dial Transplant, 2025, 41(1): 7-9. DOI: 10.1093/ndt/gfaf177.

[2] Yang K, Fang Y Y, He J B, *et al*. Prevalence and risk factors of depression in patients with diabetes mellitus: a systematic review and meta-analysis [J]. Front Endocrinol, 2025, 16: 1660478. DOI: 10.3389/fendo.2025.1660478.

[3] Toda T, Parylak S L, Linker S B, *et al*. The role of adult hippocampal neurogenesis in brain health and disease [J]. Mol Psychiatry, 2019, 24(1): 67-87. DOI: 10.1038/s41380-018-0036-2.

[4] Ma Y, Wang M Y, Zhang M Y, *et al*. Protective effect of Nootkatone against hippocampal injury in diabetes mellitus with depression *via* PI3K/AKT pathway: Network pharmacology, molecular docking and experimental validation [J]. Eur J Pharmacol, 2026, 1017: 178650. DOI: 10.1016/j.ejphar.2026.178650.

[5] Wu S X, Liang X L, Zhu Q Q, *et al*. Altered hippocampal subfield volumes are associated with memory and executive function in patients with type 2 diabetes mellitus [J]. World J Diabetes, 2025, 16(7): 104424. DOI: 10.4239/wjd.v16.i7.104424.

[6] Sun F F, Shuai Y F, Wang J R, *et al*. Hippocampal gray matter volume alterations in patients with first-episode and recurrent major depressive disorder and their associations with gene profiles [J]. BMC Psychiatry, 2025, 25(1): 134. DOI: 10.1186/s12888-025-06562-4.

[7] Barde Y A. The physiopathology of brain-derived neurotrophic factor [J]. Physiol Rev, 2025, 105(4): 2073-2140. DOI: 10.1152/physrev.00038.2024.

[8] Zhang K Q, Zhai W H, Ge X L, *et al*. Targeting BDNF with acupuncture: a novel integrated strategy for diabetes and depression comorbidity [J]. Heliyon, 2023, 9(12): e22798. DOI: 10.1016/j.heliyon.2023.e22798.

[9] Mather S, Fisher P, Nevitt S, *et al*. The limited efficacy of psychological interventions for depression in people with type 1 or type 2 diabetes: an individual participant data meta-analysis (IPD-MA) [J]. J Affect Disord, 2022, 310: 25-31. DOI: 10.1016/j.jad.2022.04.132.

[10] Hoogendoorn C J, Krause-Steinrauf H, Uschner D, *et al*. Emotional distress predicts reduced type 2 diabetes treatment adherence in the glycemia reduction approaches in diabetes: a comparative effectiveness study (GRADE) [J]. Diabetes Care, 2024, 47(4): 629-637. DOI: 10.2337/dc23-1401.

[11] Zheng M N, Yu H, Xue Y, *et al*. The protective effect of hydrogen-rich water on rats with type 2 diabetes mellitus[J]. Mol Cell Biochem, 2021, 476(8): 3089-3097. DOI: 10.1007/s11010-021-04145-x.

[12] Satoh Y, Araki Y, Kashitani M, *et al*. Molecular hydrogen prevents social deficits and depression- like behaviors induced by low-intensity blast in mice[J]. J Neuropathol Exp Neurol, 2018, 77(9): 827-836. DOI: 10.1093/jnen/nly060.

[13] Shayea A M F, Renno W M, Qabazard B, *et al*. Neuroprotective effects of a hydrogen sulfide donor in streptozotocin-induced diabetic rats[J]. Int J Mol Sci, 2023, 24(23): 16650. DOI: 10.3390/ijms242316650.

[14] Fan H J, Shi Y H, Liu H Q, *et al*. Inhalation of H2/O2 (66.7%/33.3%) mitigates depression-like behaviors in diabetes mellitus complicated with depression mice *via* suppressing inflammation and preventing hippocampal damage[J]. Biomed Pharmacother, 2024, 180: 117559. DOI: 10.1016/j.biopha.2024.117559.

[15] Nabavi S F, Sobarzo-Sánchez E, Nabavi S M, *et al*. Bi-3-azaoxoisoaporphine derivatives have antidepressive properties in a murine model of post stroke-depressive like behavior[J]. Curr Neurovasc Res, 2013, 10(2): 164-171. DOI: 10.2174/1567202611310020009.

[16] Yu H L, Deng X Q, Li Y J, *et al*. N-palmitoylethanolamide, an endocannabinoid, exhibits antidepressant effects in the forced swim test and the tail suspension test in mice[J]. Pharmacol Rep, 2011, 63(3): 834-839. DOI: 10.1016/s1734-1140(11)70596-5.

[17] AbdElmageed R M, Mohammed Hussein S M. Risk of depression and suicide in diabetic patients[J]. Cureus, 2022, 14(1): e20860. DOI: 10.7759/cureus.20860.

[18] 杜青, 赵洪庆, 王宇红, 等. 左归降糖解郁方对糖尿病并发抑郁症模型大鼠海马神经元突触功能可塑性相关蛋白的影响[J]. 中国现代应用药学, 2016, 33(5): 521-525. DOI: 10.13748/j.cnki.issn1007-7693.2016.05.001.
Du Q, Zhao H Q, Wang Y H, *et al*. Effects of Zuogui Jiangtang Jieyu Fang on synaptic function plasticity in hippocampal neurons of diabetes mellitus with depression model rats[J]. Chin J Mod Appl Pharm, 2016, 33(5): 521-525. DOI: 10.13748/j.cnki.issn1007-7693.2016.05.001.

[19] Sun B, Jia X Y, Yang F, *et al*. CREB-mediated generation and neuronal growth regulates the behavioral improvement of geniposide in diabetes-associated depression mouse model[J]. Neurosci Res, 2021, 165: 38-44. DOI: 10.1016/j.neures.2020.05.003.

[20] Furman B L. Streptozotocin-induced diabetic models in mice and rats[J]. Curr Protoc, 2021, 1(4): e78. DOI: 10.1002/cpz1.78.

[21] Lee J W, Lee Y K, Yuk D Y, *et al*. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation[J]. J Neuroinflammation, 2008, 5: 37. DOI: 10.1186/1742-2094-5-37.

[22] Couch Y, Trofimov A, Markova N, *et al*. Low-dose lipopolysaccharide (LPS) inhibits aggressive and augments depressive behaviours in a chronic mild stress model in mice[J]. J Neuroinflammation, 2016, 13(1): 108. DOI: 10.1186/s12974-016-0572-0.

[23] Yin R, Zhang K L, Li Y M, *et al*. Lipopolysaccharide-induced depression-like model in mice: meta-analysis and systematic evaluation[J]. Front Immunol, 2023, 14: 1181973. DOI: 10.3389/fimmu.2023.1181973.

[24] Chong Z Z, Souayah N. Neuroinflammation in diabetic peripheral neuropathy and therapeutic implications[J]. Rev Neurosci, 2025, 36(7): 749-762. DOI: 10.1515/revneuro-2025-0031.

[25] Karakula-Juchnowicz H, Rog J, Juchnowicz D, *et al*. The study evaluating the effect of probiotic supplementation on the mental status, inflammation, and intestinal barrier in major depressive disorder patients using gluten-free or gluten-containing diet (SANGUT study): a 12-week, randomized, double-blind, and placebo-controlled clinical study protocol[J]. Nutr J, 2019, 18(1): 50. DOI: 10.1186/s12937-019-0475-x.

[26] Gao Q, Song H, Wang X T, *et al*. Molecular hydrogen increases resilience to stress in mice[J]. Sci Rep, 2017, 7(1): 9625. DOI: 10.1038/s41598-017-10362-6.

[27] Zhang Y, Su W J, Chen Y, *et al*. Effects of hydrogen-rich water on depressive-like behavior in mice[J]. Sci Rep, 2016, 6: 23742. DOI: 10.1038/srep23742.

[28] Maggio N, Segal M. Steroid modulation of hippocampal plasticity: switching between cognitive and emotional memories[J]. Front Cell Neurosci, 2012, 6: 12. DOI: 10.3389/fncel.2012.00012.

[29] Hammad H, Shaltout I, Fawzy M M, *et al*. Brain-derived neurotrophic factor level and gene polymorphism as risk factors for depression in patients with type 2 diabetes mellitus-a case-controlled study[J]. Curr Diabetes Rev, 2025, 21(4): 13-20. DOI: 10.2174/0115733998274778231218145449.

[30] Lenart L, Balogh D B, Lenart N, *et al*. Novel therapeutic potential of angiotensin receptor 1 blockade in a rat model of diabetes-associated depression parallels altered BDNF signalling[J]. Diabetologia, 2019, 62(8): 1501-1513. DOI: 10.1007/s00125-019-4888-z.

[31] Zhang J C, Yao W, Hashimoto K. Brain-derived

neurotrophic factor (BDNF)-TrkB signaling in inflammation-related depression and potential therapeutic targets[J]. Curr Neuropharmacol, 2016, 14(7): 721-731. DOI: 10.2174/1570159x14666160119094646.

[32] Chen Z W, Gu J P, Lin S S, *et al*. Saffron essential oil ameliorates CUMS-induced depression-like behavior in mice *via* the MAPK-CREB1-BDNF signaling pathway [J]. J Ethnopharmacol, 2023, 300: 115719. DOI: 10.1016/j.jep.2022.115719.

[33] Wen M Y, Lian Z S, Huang L Q, *et al*. Magnetic resonance spectroscopy for assessment of brain injury in the rat model of sepsis[J]. Exp Ther Med, 2017, 14(5): 4118-4124. DOI:10.3892/etm.2017.5034.

[34] Mueller S G, Schuff N, Yaffe K, *et al*. Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease[J]. Hum Brain Mapp, 2010, 31(9): 1339-1347. DOI:10.1002/hbm.20934.

[35] Lee S, Kim T D, Kim R Y, *et al*. Hippocampal subregional alterations and verbal fluency in the early stage of type 2 diabetes mellitus[J]. Eur J Neurosci, 2021, 54(10): 7550-7559. DOI:10.1111/ejn.15505.

[36] Fotuhi M, Do D, Jack C. Modifiable factors that alter the size of the hippocampus with ageing[J]. Nat Rev Neurol, 2012, 8(4): 189-202. DOI:10.1038/nrneurol.2012.27.

[37] Sharma S, Brown C E. Microvascular basis of cognitive impairment in type 1 diabetes[J]. Pharmacol Ther, 2022, 229: 107929. DOI:10.1016/j.pharmthera.2021.107929.

[38] Sharan P, Vellapandian C. Hypothalamic-pituitary-adrenal (HPA) axis: unveiling the potential mechanisms involved in stress-induced Alzheimer's disease and depression[J]. Cureus, 2024, 16(8): e67595. DOI: 10.7759/cureus.67595.

[39] Kodl C T, Franc D T, Rao J P, *et al*. Diffusion tensor imaging identifies deficits in white matter microstructure in subjects with type 1 diabetes that correlate with reduced neurocognitive function[J]. Diabetes, 2008, 57(11): 3083-3089. DOI:10.2337/db08-0724.

[40] Müller H P, Kassubek J. Toward diffusion tensor imaging as a biomarker in neurodegenerative diseases: technical considerations to optimize recordings and data processing [J]. Front Hum Neurosci, 2024, 18: 1378896. DOI: 10.3389/fnhum.2024.1378896.

(**收稿**:2026-04-01;**修回**:2026-07-30)

(编辑 刘秋池)