论著·神经科学

Mdivi-1通过抑制过度线粒体自噬改善低氧复合丙泊酚引起的未成熟SD大鼠认知功能障碍

徐 瑶,杨 顺,杨 飞,杨 颖,云焕杰,Tauseef Ahmad,涂生芬 重庆医科大学附属儿童医院麻醉科,国家儿童健康与疾病临床医学研究中心,儿童发育疾病研究教育部重点实验室,儿童神经发育与认知障碍重庆市重点实验室,重庆

［**摘要**］ **目的** 探讨线粒体分裂抑制剂1(mitochondrial division inhibitor 1,Mdivi-1)对低氧复合丙泊酚麻醉引起的未成熟SD大鼠认知功能障碍的保护作用。**方法** 将72只7日龄SD大鼠[雌雄数量均等,体质量(16.14±3.08)g]按照随机数字表法分为(n=18):空白对照组(CON组)、抑制剂空白对照组(MCON组)、丙泊酚低氧组(PH组)、抑制剂丙泊酚低氧组(MPH组)。提前30 min对MCON组和MPH组分别给予Mdivi-1(2.4 mg/kg)预处理,PH组和MPH组分别给予丙泊酚(50 mg/kg),腹腔注射完成后,将其立即转移至氧浓度为18%的恒温箱内,持续监测直至动物恢复翻正反射后转入常氧环境中饲养,上述操作每天1次,连续7 d。转录组测序筛选差异表达基因(differential expressed genes, DEGs),京都基因与基因组百科全书(Kyoto encyclopedia of genes and genomes,KEGG)富集分析对其进行功能分析;Morris水迷宫行为学试验检测空间学习和记忆功能;HE染色观察海马CA1区神经元形态学变化;透射电镜观察海马神经元内线粒体形态改变;Western blot检测海马组织中Parkin泛素蛋白连接酶(Parkin Rbr E3 ubiquitin protein ligase, Parkin)、PTEN诱导假定激酶1(PTEN-induced putative kinase 1, PINK1)蛋白表达情况。**结果** 转录组测序KEGG分析结果:与CON组相比,PH组线粒体自噬通路显著富集。Morris水迷宫试验结果显示:与CON组和MPH组相比,PH组大鼠逃逸潜伏期延长(P<0.05),穿越平台次数减少(P<0.05),目标象限停留时间缩短(P<0.01)。HE染色结果显示:PH组细胞排列紊乱无序,部分区域出现细胞数量锐减的丢失现象,细胞间隙显著增宽;MPH组细胞排列虽也表现出稀疏状态,但相较于PH组,其程度较轻,细胞间隙仅呈现轻微增宽;CON组与MCON组的细胞形态结构良好,轮廓较为清晰,细胞核大小均匀一致,形状均呈圆形。透射电镜结果显示:PH组海马神经元内线粒体肿胀、变形,线粒体嵴紊乱、溶解、消失;MPH组仅部分线粒体肿胀、嵴减少;CON组和MCON组线粒体形态相对完整,基质均匀。Western blot检测结果显示:与CON组相比,PH组SD大鼠海马组织中Parkin、PINK1表达上调(P<0.05);与PH组相比,MPH组SD大鼠海马组织中Parkin、PINK1表达下调(P<0.05)。**结论** Mdivi-1可能通过抑制过度线粒体自噬减轻海马神经元损伤进而改善低氧复合丙泊酚引起的未成熟SD大鼠认知功能障碍。

［**关键词**］ Mdivi-1;丙泊酚;低氧;线粒体自噬;认知功能障碍
［**中图法分类号**］ R619;R741;R977.6 ［**文献标志码**］ A

Mdivi-1 improves cognitive dysfunction in immature SD rats induced by hypoxia combined with propofol by inhibiting excessive mitophagy

XU Yao, YANG Shun, YANG Fei, YANG Ying, YUN Huanjie, TAUSEEF Ahmad, TU Shengfen Department of Anesthesiology Children's Hospital of Chongqing Medical University, National Clinical Research Center for Child Health and Disorders, Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing Key Laboratory of Child Neurodevelopment and Cognitive Disorders, Chongqing, China

［**Abstract**］ Objective To investigate the protective effect of mitochondrial division inhibitor 1 (Mdivi-1),

［**基金项目**］ 国家儿童健康与疾病临床医学研究中心临床医学研究一般项目(NCRCCHD-2022-GP-0X);重庆市自然科学基金面上项目(CSTB2023NSCQ-MSX0434)
［**通信作者**］ 涂生芬, E-mail: 15213324272@163.com

2097-0927/Copyright © 2025, 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:徐瑶,杨顺,杨飞,等. Mdivi-1通过抑制过度线粒体自噬改善低氧复合丙泊酚引起的未成熟SD大鼠认知功能障碍[J]. 陆军军医大学学报,2025,47(21):2621-2629. DOI:10.16016/j.2097-0927.202505075.
Citation:XU Y, YANG S, YANG F, *et al*. Mdivi-1 improves cognitive dysfunction in immature SD rats induced by hypoxia combined with propofol by inhibiting excessive mitophagy[J]. J Army Med Univ,2025,47(21):2621-2629. DOI:10.16016/j.2097-0927.202505075.

a mitophagy inhibitor, on cognitive dysfunction in immature SD rats induced by hypoxia combined with propofol. **Methods** A total of 72 neonatal SD rats (half male and half female, aged 7 d, weighing 16.14±3.08 g) were randomly divided into 4 groups (n=18): blank control group (CON), Mdivi-1+blank control group (MCON), propofol+hypoxia group (PH), and Mdivi-1+propofol+ hypoxia group (MPH). The MCON group and MPH group were pretreated with 2.4 mg/kg Mdivi-1 in 30 min in advance, and subsequently, 50 mg/kg propofol was respectively given to the PH group and the MPH group. Immediately after the intraperitoneal injections were completed, the corresponding rats were transferred into a constant temperature chamber containing 18%O_2. Then the rats were continuously monitored until resuming the reversing reflex and then transferred to a normoxic environment. The hypoxic treatment was carried out once per day for 7 consecutive days. Transcriptome sequencing was used to screen for differential expressed genes (DEGs), and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis was employed to conduct functional analysis. Morris water maze test was applied to exam spatial learning and memory abilities. HE staining was used to observe the morphological changes of neurons in the hippocampal CA1 region. Morphological changes of mitochondria in hippocampal neurons were observed by transmission electron microscopy (TEM). Western blotting was utilized to detect the expression of Parkin Rbr E3 ubiquitin protein ligase (Parkin) and PTEN-induced putative kinase 1 (PINK1) in the hippocampal tissues. **Results** KEGG analysis results indicated that the mitophagy pathway was more significantly enriched in the PH group when compared with the CON group. Morris water maze test revealed that the PH group exhibited obviously prolonged escape latency (P<0.05), reduced frequency of crossing the platform (P<0.05), and shortened residence time in the target quadrant (P<0.01) when compared with the CON group and the MPH group. HE staining demonstrated that the cells in the PH group were in disordered arrangement, with great loss in cell count in some areas, and widened intercellular spaces. Although the cell arrangement in the MPH group also showed a sparse state, but the condition was milder than that in the PH group, with slightly widened intercellular space. In the CON group and the MCON group, the cells exhibited round in shape, good morphological structure, relatively clear contours, and uniform cell nuclei. TEM revealed that in the hippocampal neurons of the PH group, the mitochondria were swollen and deformed, and mitochondrial cristae were disordered, dissolved and disappeared. In the MPH group, only some mitochondria were swollen and the cristae were reduced. However, the mitochondrial morphology of the CON and MCON groups was relatively complete, and the matrix was uniform. Western blot analysis showed that the expression levels of Parkin and PINK1 in the hippocampus were up-regulated in the PH group than the CON group (P<0.05), and reduced in the MPH group than the PH group (P<0.05). **Conclusion** Mdivi-1 may reduce hippocampal neuron damage by inhibiting excessive mitophagy and then improve cognitive dysfunction in immature SD rats induced by hypoxia combined with propofol.

[**Key words**] Mdivi-1; propofol; hypoxia; mitophagy; cognitive dysfunction

Supported by the General Project of Clinical Medical Research From National Clinical Research Center for Child Health and Disorders (NCRCCHD-2022-GP-0X) and the General Project of Natural Science Foundation of Chongqing (CSTB2023NSCQ-MSX0434). Corresponding author: TU Shengfen, E-mail: 15213324272@163.com

术后认知功能障碍(postoperative cognitive dysfunction,POCD)是麻醉手术后常见的神经认知相关并发症,其临床特征主要表现为术后出现的记忆功能减退、注意力障碍、执行功能受损以及语言能力下降等认知功能的可逆性或持续性损害[1]。根据现有研究证据,POCD的发生机制涉及多因素交互作用,其中主要危险因素包括高龄(>65岁)、术前合并基础疾病(如高血压、糖尿病等)、麻醉药物(特别是吸入性麻醉剂和苯二氮䓬类药物)的神经毒性作用、围术期低氧血症、短期内重复麻醉暴露以及术后感染等[2]。丙泊酚作为围术期常用的静脉全身麻醉药,被广泛应用于临床,但其对认知功能的影响仍存在学术争议[3]。值得注意的是,合并有组织氧合障碍基础疾病的患儿(如紫绀型先天性心脏病以及先天性膈疝等),常处于持续性低氧血症的病理生理状态,临床实践中发现这些患儿在接受反复多次全身麻醉后,其学习和记忆能力会受

到影响,且呈现病程迁延、损伤程度不一的特征[4]。

本课题组前期通过构建未成熟SD大鼠双模型研究体系(体内发育期动物模型与体外HT22海马神经元培养模型),首次发现重复丙泊酚暴露对伴有低氧血症的发育期SD大鼠可造成显著的远期空间记忆与学习能力损害[5],通过RNA测序(RNA sequencing, RNA-seq)进行差异表达基因的筛选,京都基因与基因组百科全书(Kyoto encyclopedia of genes and genomes, KEGG)信号通路分析结果显示线粒体自噬通路显著富集。因此提出科学假设,该过程可能涉及线粒体质量控制失衡-线粒体自噬的异常激活会通过PTEN诱导假定激酶1(PTEN-induced putative kinase 1, PINK1)/Parkin泛素蛋白连接酶(Parkin Rbr E3 ubiquitin protein ligase, Parkin)依赖性途径诱导线粒体过度分裂,进而导致海马神经元突触可塑性损伤[6]。海马是缺血缺氧、神经变性疾病及药物相关损伤的主要部位,在学习、记忆及情绪反应等众多方面发挥着重要作用[7]。线粒体分裂抑制剂1(mitochondrial division inhibitor 1,Mdivi-1)是一种特异的线粒体分裂抑制剂,通过干扰线粒体分裂相关蛋白1(dynamin-related protein 1, Drp1)的GTP酶活性,阻止线粒体的分裂过程,从而使线粒体趋于融合状态[8]。课题组前期研究结果表明,经Mdivi-1处理的HT22海马神经元细胞,可以有效缓解低氧复合丙泊酚引起的线粒体形态和功能异常[9],然而该抑制剂对低氧复合丙泊酚暴露的未成熟SD大鼠认知功能的影响尚未明确。基于此,本研究拟从线粒体自噬调控机制入手,系统地探究Mdivi-1对低氧复合丙泊酚引起的未成熟SD大鼠认知功能障碍的干预作用。

1 材料与方法

1.1 材料与试剂

丙泊酚注射液购自英国阿斯利康制药公司;Mdivi-1、HRP标记山羊抗兔IgG、BCA试剂盒购自上海碧云天生物技术有限公司;多聚甲醛、磷酸盐缓冲液PBS购自北京兰杰柯科技有限公司;2.5%戊二醛电镜液购自武汉赛维尔生物科技有限公司;全蛋白提取试剂盒购自江苏凯基生物技术股份有限公司;10% PAGE凝胶快速制备试剂盒购自上海雅酶生物医药科技有限公司;Anti-Parkin、Anti-PINK1购自武汉三鹰生物技术公司;鼠源GAPDH抗体购自成都正能生物技术有限责任公司;HRP标记山羊抗鼠IgG购自苏州百远生物科技有限公司。

1.2 实验动物与建模

选取72只7日龄SPF级SD大鼠,雌雄数量均等,体质量(16.14±3.08)g,均由重庆医科大学动物实验中心供应,且具备动物实验合格资质,动物实验均通过重庆医科大学附属儿童医院实验动物福利伦理审查委员会批准(CHCMU-IACUC20240617003)。饲养环境严格遵循标准条件,环境温度保持在(25±2)℃,相对湿度控制在55%±5%,同时实施12 h光照与12 h黑暗交替的昼夜节律。

1.2.1 动物分组 将72只SD大鼠采用随机数字表法分为(n=18):空白对照组(CON组)、抑制剂空白对照组(MCON组)、丙泊酚低氧组(PH组)、抑制剂丙泊酚低氧组(MPH组)。

1.2.2 干预措施 提前30 min对MCON组和MPH组分别给予Mdivi-1(2.4 mg/kg)腹腔注射预处理;PH组和MPH组分别给予丙泊酚(50 mg/kg)腹腔注射,完成腹腔注射后,将大鼠立即转移至氧浓度为18%的恒温箱内(定制混合气体瓶,CY-12C测氧仪监测氧浓度),持续监测直至动物恢复翻正反射后转入常氧环境中饲养,上述操作每天1次,连续7 d。

1.2.3 取材和标本制备 干预结束后24 h内,各组随机抽取9只SD大鼠,给予戊巴比妥钠(50 mg/kg)腹腔注射麻醉处理后,于冰上快速断头,精准剥离双侧海马组织,保存于-80 ℃环境下,便于后续进行相关指标检测。各组另随机抽取3只SD大鼠,给予戊巴比妥钠(50 mg/kg)腹腔注射麻醉处理后,使用0.1 mol/L PBS(pH=7.4)和4%多聚甲醛经心脏灌注,解剖并分离脑组织,浸泡于4%多聚甲醛固定液用于后续相关指标检测。其余大鼠待其生长至28 d后进行Morris水迷宫试验。

1.3 方法

1.3.1 转录组测序 研究初期,取CON组和PH组SD大鼠新鲜海马组织,在Illumina NovaSeq 6000平台上进行RNA-seq分析(oebiotech,中国),以筛选差异表达基因。使用KEGG富集分析对差异表达基因(differential expressed genes, DEGs)进行功能分析,以2为底取对数得到差异倍数(fold change, FC)对数折叠变化,DEGs的筛选条件为$|\mathrm{Log_2\,FC}|\geq 0.585$且$P<0.05$。

1.3.2 Morris水迷宫试验 每组取6只幼鼠饲养至28日龄,开始进行Morris水迷宫试验,正式开展测试的前1 d,设定为适应期,让每只大鼠在圆形水池中自由游泳60 s,帮助其熟悉实验环境,减少因环境陌生带来的应激反应。

定位航行实验:第1~5天,将大鼠头朝池壁放入水

中。使用计时设备精确记录大鼠在60 s内成功寻找到隐藏平台所需的时间,即逃逸潜伏期,若大鼠在60 s内未找到平台,则将其引导至平台并停留10 s,该大鼠的逃逸潜伏期记录为60 s。每只大鼠每天进行4次游泳实验,每次从水池的不同象限入水,且相邻2次实验之间的间隔时间设定为15 min。

空间探索实验:第6天撤除水池中的平台,将大鼠从原平台对侧象限放入水中,记录60 s内穿越原平台所在位置的次数,以及在目标象限(第3象限)停留的时间。在整个测试过程中,严格控制实验室内环境条件,保持灯光亮度、物品摆放等室内环境因素一致。

1.3.3 HE染色 采用4%多聚甲醛溶液对全脑组织予以固定,脱水后用石蜡进行包埋,制备成厚度为4 μm的组织切片。将切片置于二甲苯中进行2次脱蜡处理,每次处理10 min;使用梯度乙醇(由高浓度至低浓度)进行脱蜡,每次处理3 min,用蒸馏水充分洗净切片,并沥干残留水分。利用苏木精对切片进行染色5 min,蒸馏水冲洗2 min,在光学显微镜下初步观察细胞核的染色深度。将切片快速置于1%盐酸乙醇溶液中进行分化处理,时间控制在3~5 s,完成后再次用蒸馏水洗涤2 min。放入饱和碳酸锂溶液浸泡5~10 s,经蒸馏水充分漂洗后沥除残留液体,再进行伊红染色。将切片放入梯度乙醇进行脱水处理,再放入二甲苯中浸泡2次,各1 min。采用中性树脂作为封固介质,待封片自然风干后,在光学显微镜下仔细观察海马神经元的受损情况。

1.3.4 透射电镜检测 冰上剥离海马组织,取海马CA1区组织1 mm^3置于2.5%戊二醛电镜液中,室温避光固定2 h,再转移至4 ℃保存运输。使用1%锇酸避光固定2 h,PBS漂洗3次,每次10 min,室温酒精梯度脱水,环氧树脂渗透包埋,切片,染色。透射电镜观察海马神经元内线粒体形态变化。

1.3.5 Western blot检测Parkin、PINK1蛋白表达 分别取各组海马组织,使用全蛋白提取试剂盒,加入裂解液于冰上裂解,使用BCA试剂盒测定蛋白浓度,煮沸变性。使用10%PAGE凝胶快速制备试剂盒,取30 μg蛋白样品,在Tris-甘氨酸缓冲系统中,以恒定电压80 V电泳分离等量的蛋白质,以恒定电流400 mA进行湿转,将蛋白质转移到0.2 μm PVDF膜上,5%脱脂奶粉摇床封闭120 min。膜用TBST缓冲液洗涤3次,4 ℃摇床孵育一抗过夜:Parkin(1∶2 500)、PINK1(1∶1 000)、GAPDH(1∶5 000)。次日TBST缓冲液洗膜3次后室温摇床孵育二抗:山羊抗鼠IgG抗体(1∶10 000)、山羊抗兔IgG抗体(1∶3 000)100 min。采用增强化学发光法(enhanced chemiluminescence,ECL)进行蛋白信号显影,使用Image Lab专业分析软件对印迹图像进行数字化处理及定量分析。目的蛋白与内参GAPDH灰度值的比值作为目的蛋白表达量。

1.4 统计学分析

采用GraphPad Prism 8.0.2进行统计分析并绘图。每个实验至少进行3次重复,计量资料以$\bar{x}\pm s$表示,多组之间的差异采用单因素方差分析(ANOVA)进行比较,并使用Tukey多重比较检验,$P<0.05$为差异具有统计学意义。

2 结果

2.1 转录组测序结果分析

利用RNA-seq技术筛选差异基因,并对其进行KEGG信号通路分析后发现:与CON组相比,低氧复合丙泊酚麻醉处理的PH组大鼠在线粒体自噬通路上呈现显著富集(图1)。

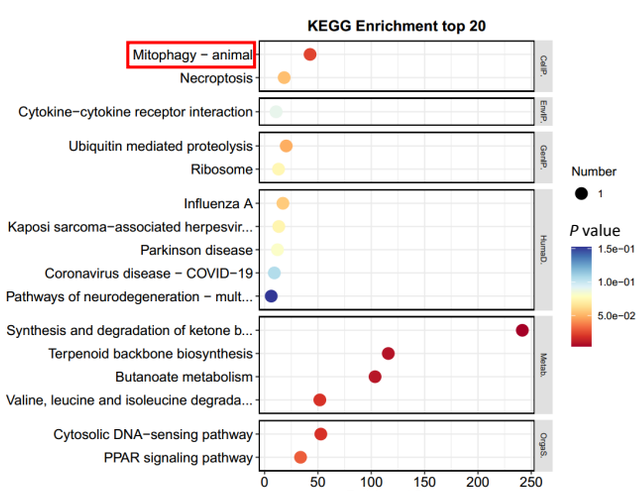


图1 基于海马组织RNA-seq的KEGG分析结果

Figure 1 KEGG analysis results based on RNA-seq in hippocampal tissue

2.2 Mdivi-1对未成熟SD大鼠空间学习和记忆能力的影响

在定位航行实验和空间探索实验期间,利用分析系统记录每只SD大鼠在水迷宫中的轨迹,同时记录其逃逸潜伏期,并统计各组大鼠穿越平台次数和在目标象限的停留时间。结果显示:在定位航行实验的第5天,与CON组和MPH组相比,PH组大鼠逃逸潜伏期延长($P<0.05$),学习能力较弱;CON组与MCON组比较无显著性差异。在空间探索实验中,与CON组和MPH组相比,PH组大鼠穿越平台次数减少($P<0.05$),目标象限停留时间缩短($P<0.01$),记忆能力较差;CON组与MCON组比较无显著性差异(图2)。

2.3　Mdivi-1对未成熟SD大鼠海马组织CA1区神经元细胞的影响

HE染色后光学显微镜下观察海马组织CA1区锥体细胞形态学改变,结果显示:PH组细胞呈现显著的病理改变,其细胞排列紊乱无序,分布极为稀疏,部分区域出现细胞数量锐减的丢失现象,且细胞间隙显著增宽;MPH组细胞排列虽也表现出稀疏状态,但相较于PH组,其程度较轻,细胞间隙仅呈现轻微增宽;而CON组与MCON组的细胞形态结构良好,轮廓较为清晰,细胞核大小均匀一致,形状均呈圆形;经统计分析,与CON组和MPH组相比,PH组大鼠海马神经细胞数量明显减少($P<0.01$);CON组与MCON组比较无显著性差异(图3)。

2.4　Mdivi-1对未成熟SD大鼠海马神经元内线粒体形态的影响

透射电镜结果显示:PH组海马神经元内线粒体肿胀、变形,线粒体嵴紊乱、溶解、消失;MPH组仅部分线粒体肿胀、嵴减少;而CON和MCON组线粒体形态相对完整,基质均匀(图4)。

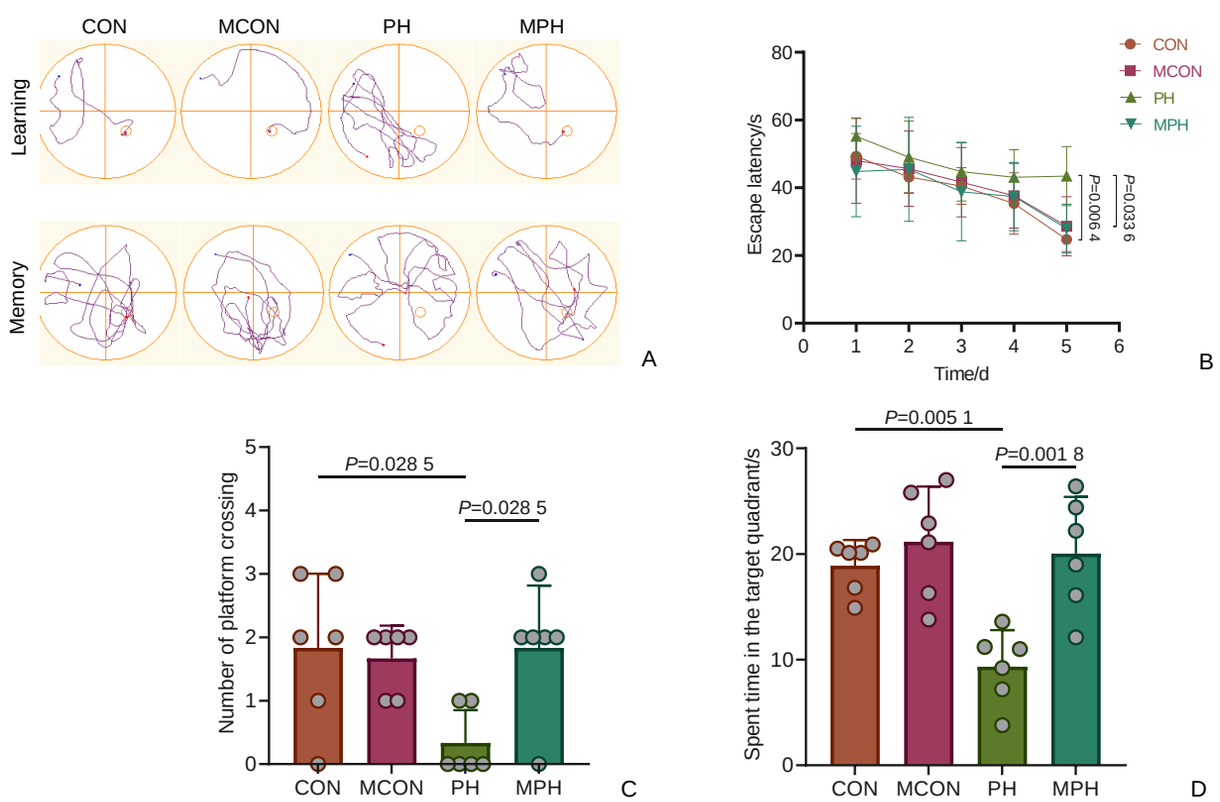
2.5　Mdivi-1对未成熟SD大鼠海马组织线粒体自噬相关蛋白表达水平的影响

通过Western blot检测Parkin、PINK1表达水平,结果显示:与CON组相比,PH组SD大鼠海马组织中Parkin、PINK1表达上调($P<0.05$);与PH组相比,经Mdivi-1预处理的MPH组SD大鼠海马组织中Parkin、PINK1表达下调($P<0.05$);与CON组相比,MCON组SD大鼠海马组织中Parkin、PINK1表达无明显差异;表明Mdivi-1有效抑制了低氧复合丙泊酚引起的异常线粒体自噬(图5)。

3　讨论

3.1　丙泊酚麻醉与认知

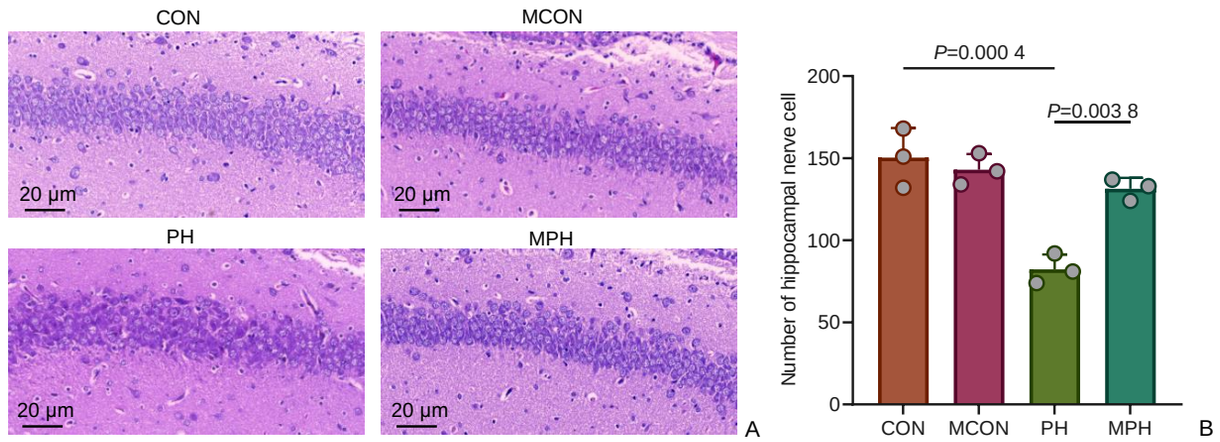
儿童由于其发育的特殊性,在进行手术时通常需要全身麻醉,而丙泊酚因其起效快、不良反应少、持续输注无明显积累等优点,成为儿童麻醉诱导与维持的首选静脉麻醉药。一些研究证实丙泊酚可以通过抑制氧化应激和炎症、稳定钙超载等方式对认知功能起到保护作用[10-11]。然而,也有一些研究认为丙泊酚会



A: Representative movement trajectory of the learning and memory phases in Morris water maze test; B: Intergroup comparison of escape latency in rats; C: Intergroup comparison of number of platform crossings in rats; D: Intergroup comparison of time spenting in the target quadrant in rats

图2　Mdivi-1预处理对大鼠空间学习和记忆能力的影响($n=6,\bar{x}\pm s$)

Figure 2　Effects of Mdivi-1 pretreatment on spatial learning and memory abilities in rats ($n=6,\bar{x}\pm s$)



A: Changes in the hippocampal CA1 region by HE staining; B: Intergroup comparison of number of hippocampal nerve cells (n=3, $\bar{x}\pm s$)

图3 Mdivi-1预处理对大鼠海马组织CA1区的影响

Figure 3 Effects of Mdivi-1 pretreatment on the hippocampal CA1 region in rats

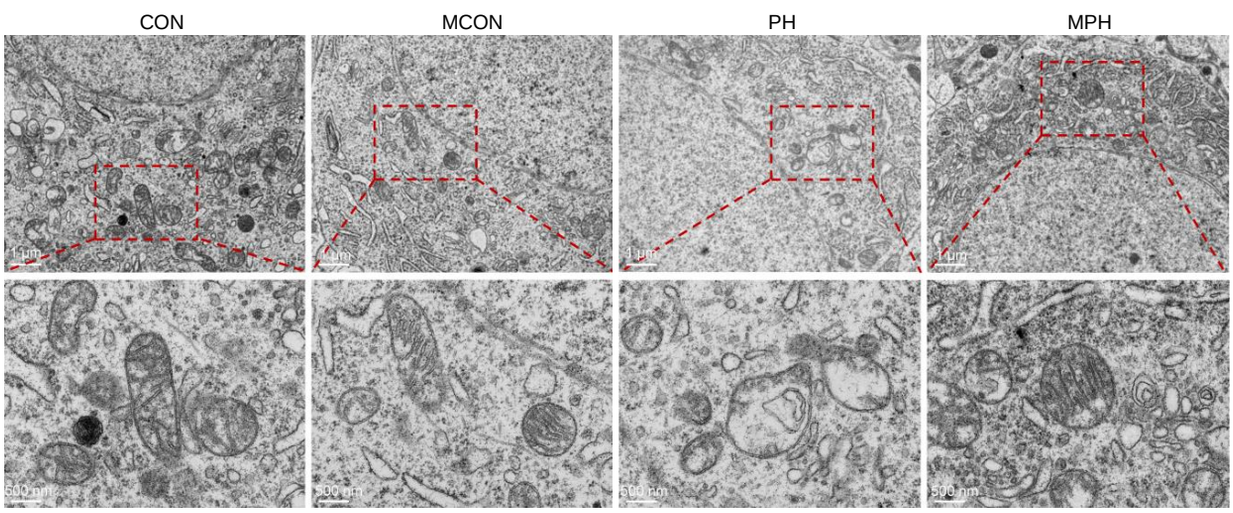
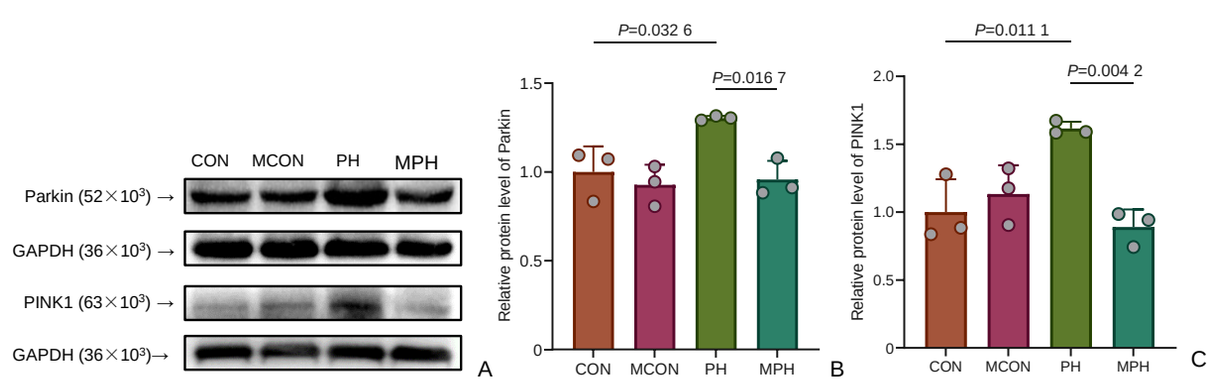


图4 透射电镜观察大鼠海马神经元内线粒体形态

Figure 4 Mitochondrial morphology within hippocampal neurons in rats by transmission electron microscopy



A: Western blotting of Parkin and PINK1 in the hippocampal tissue in rats; B and C: Statistical analysis of relative expression levels of Parkin and PINK1 in the hippocampal tissue in rats

图5 Mdivi-1预处理对各组大鼠海马组织线粒体自噬相关蛋白表达的影响(n=3, $\bar{x}\pm s$)

Figure 5 Effects of Mdivi-1 pretreatment on the expression of mitophagy-related proteins in the hippocampal tissues of rats (n=3, $\bar{x}\pm s$)

引起术后认知功能障碍[12-13]。这种矛盾的结果可能与年龄、是否合并基础疾病、丙泊酚剂量、其他麻醉药物、反复多次麻醉等因素有关。因此,本课题组前期构建了低氧复合丙泊酚的SD大鼠模型,发现低氧复合丙泊酚麻醉会引起未成熟SD大鼠认知功能障碍[14-16]。本研究中Morris水迷宫试验同样提示:与CON组相比,PH组SD大鼠第5天逃逸潜伏期延长($P<0.01$),穿越平台次数减少($P<0.05$),目标象限停留时间缩短($P<0.01$),该结果与前期研究一致。

3.2 低氧复合丙泊酚与线粒体自噬

基于上述发现,课题组进一步采用RNA-seq技术,对SD大鼠的海马组织进行了KEGG分析,结果表明线粒体自噬可能参与其中。线粒体自噬是一种选择性自噬过程,用于清除细胞内功能失调或多余的线粒体,维持细胞内线粒体的活性和数量的平衡,从而确保细胞的正常功能[17]。在这个过程中,PINK1和Parkin发挥关键作用[18-19]。线粒体一直处于分裂和融合的动态平衡中,但有缺陷的和过度激活的线粒体自噬会使线粒体功能异常[20]。一些研究证实,丙泊酚的神经毒性影响可能与线粒体功能异常有关[21-22];而在课题组前期研究中也发现,经低氧复合丙泊酚处理的HT22细胞会显著促进线粒体自噬,引起线粒体形态异常和功能障碍[9]。在本研究中,通过动物体内实验得出同样的结论:Western blot结果提示与CON组相比,PH组与线粒体自噬相关的典型蛋白Parkin、PINK1表达明显上调($P<0.05$);透射电镜结果显示PH组海马神经元内线粒体肿胀、变形,线粒体嵴紊乱、溶解、消失;HE染色结果显示PH组海马组织CA1区细胞排列紊乱无序,分布稀疏,部分区域出现细胞数量锐减的丢失现象,细胞间隙显著增宽,说明低氧复合丙泊酚会激活过度的线粒体自噬,进而导致海马神经元的损伤。

3.3 Mdivi-1的保护效应

Mdivi-1是一种能够穿越细胞膜的喹唑酮类化合物,通过抑制线粒体分裂,进一步阻断异常的线粒体自噬[23]。虽然Mdivi-1对线粒体动力学的抑制作用仍然存在争议[24],但其在病理模型中的治疗价值已通过多系统研究获得验证,包括阿尔茨海默病[25]、急性心肌梗死[26]、缺血再灌注损伤[27]。国内学者也进一步拓展其应用场景,证明Mdivi-1具有髓鞘保护[28]、改善肝性脑病[29]、肠道屏障功能保护[30]等作用。因此,本研究在发育期SD大鼠模型中同样验证了Mdivi-1(2.4 mg/kg腹腔注射,术前30 min给药)[31]的保护效应:①认知行为学层面,Morris水迷宫结果显示,与PH组比较,MPH组第5天逃逸潜伏期缩短($P<0.05$),目标象限停留时间延长、穿越平台次数增多($P<0.05$);②组织病理学层面,PH组呈现显著的病理改变,其细胞排列紊乱无序,分布极为稀疏,部分区域出现细胞数量锐减的丢失现象,细胞间隙显著增宽;MPH组细胞排列虽也表现出稀疏状态,但相较于PH组,其程度较轻,细胞间隙仅呈现轻微增宽;透射电镜结果显示:PH组海马神经元内线粒体肿胀、变形,线粒体嵴紊乱、溶解、消失;MPH组仅部分线粒体肿胀、嵴减少;③分子机制层面,Western blot检测显示,与PH组比较,MPH组海马组织中线粒体自噬相关的典型蛋白Parkin、PINK1表达明显下调($P<0.05$)。这些数据证实,通过药理学调控线粒体分裂/融合平衡,可有效阻断低氧复合丙泊酚协同作用引发的过度线粒体自噬级联反应,进而改善发育期神经系统的认知功能结局。胡锦涛等[32]的研究同样发现Mdivi-1可以抑制线粒体过度分裂及降低线粒体通透性,并且上调BDNF和Bcl-2,从而减轻丙泊酚诱导的发育期海马神经元凋亡,该结论与本研究具有一致性。

3.4 本研究的局限性

本研究是对前期研究的回复性验证,仍存在以下需要完善的关键科学问题:①机制特异性验证不足,未通过Drp1 GTP酶活性检测和线粒体功能实时监测直接证明Mdivi-1的神经保护效应与Drp1功能抑制的剂量-效应关系;②上游调控网络解析缺失,RNA-seq分析结果还提示Depp1基因为低氧丙泊酚组与其对照组之间的显著差异基因,最新研究表明Depp1是参与自噬激活的主要缺氧诱导因子,ROS和缺氧是Depp1表达的主要刺激因素,在应激条件下调节线粒体自噬[33],然而Depp1是否作为线粒体自噬的上游靶点对其进行调控仍需要进一步的实验进行验证;③临床转化具有挑战性,目前仍然缺乏对于Mdivi-1血脑屏障穿透率的研究,尤其在正常生理状态下难以达到有效脑内浓度,仅有部分间接证据支持[34]。同时Mdivi-1可能会通过抑制复合体Ⅰ(呼吸链关键部分),导致ATP合成效率下降、生成过量ROS,长期可能引起神经元损伤[35]。因此对于Mdivi-1的临床价值需要更谨慎的评估,以平衡疗效与风险。

综上所述,本研究结果表明Mdivi-1可以减轻海马神经元损伤进而改善低氧复合丙泊酚引起的未成熟SD大鼠认知功能障碍,其机制可能与Mdivi-1抑制过度的线粒体自噬有关。该研究结论有望为临床防治小儿术后认知功能障碍提供理论依据和创新性治疗思路。

利益冲突 本文所有作者声明不存在利益冲突

参考文献:

[1] WU W F, CHEN C, LIN J T, *et al*. Impaired synaptic plasticity and decreased glutamatergic neuron excitability induced by SIRT1/BDNF downregulation in the hippocampal CA1 region are involved in postoperative cognitive dysfunction [J]. Cell Mol Biol Lett, 2024, 29(1): 79. DOI: 10.1186/s11658-024-00595-5.

[2] CZYŻ-SZYPENBEJL K, MĘDRZYCKA-DĄBROWSKA W, KWIECIEŃ-JAGUŚ K, *et al*. The occurrence of postoperative cognitive dysfunction (POCD) —systematic review [J]. Psychiatr Pol, 2019, 53(1): 145-160. DOI: 10.12740/PP/90648.

[3] LIU P F, ZHAO S, QIAO H, *et al*. Does propofol definitely improve postoperative cognitive dysfunction—a review of propofol-related cognitive impairment [J]. Acta Biochim Biophys Sin, 2022, 54(7): 875-881. DOI: 10.3724/abbs.2022067.

[4] SCHNEUER F J, BENTLEY J P, DAVIDSON A J, *et al*. The impact of general anesthesia on child development and school performance: a population-based study [J]. Paediatr Anaesth, 2018, 28(6): 528-536. DOI: 10.1111/pan.13390.

[5] LIU L, GAO W, YANG S, *et al*. Ferritinophagy-mediated hippocampus ferroptosis is involved in cognitive impairment in immature rats induced by hypoxia combined with propofol [J]. Neurochem Res, 2024, 49(7): 1703-1719. DOI: 10.1007/s11064-024-04128-6.

[6] PARK Y S, CHOI S E, KOH H C. PGAM5 regulates PINK1/Parkin-mediated mitophagy *via* DRP1 in CCCP-induced mitochondrial dysfunction[J]. Toxicol Lett, 2018, 284: 120-128. DOI: 10.1016/j.toxlet.2017.12.004.

[7] CHURILOVA A, ZACHEPILO T, BARANOVA K, *et al*. Differences in the autophagy response to hypoxia in the hippocampus and neocortex of rats[J]. Int J Mol Sci, 2022, 23(14): 8002. DOI: 10.3390/ijms23148002.

[8] SUN J, YU F, WANG T, *et al*. The role of DRP1-PINK1-Parkin-mediated mitophagy in early cadmium-induced liver damage[J]. Toxicology, 2022, 466: 153082. DOI: 10.1016/j.tox.2021.153082.

[9] YANG S, XU Y, AHMAD T, *et al*. Mdivi-1 alleviates ferroptosis induced by hypoxia combined with propofol in HT22 cells by inhibiting excessive mitophagy [J]. Mol Biol Rep, 2024, 51(1): 870. DOI: 10.1007/s11033-024-09812-y.

[10] YU S S, XIN W Q, JIANG Q L, *et al*. Propofol exerts neuroprotective functions by down-regulating microRNA-19a in glutamic acid-induced PC12 cells[J]. Biofactors, 2020, 46(6): 934-942. DOI: 10.1002/biof.1607.

[11] LI W X, LUO R Y, CHEN C, *et al*. Effects of propofol, dexmedetomidine, and midazolam on postoperative cognitive dysfunction in elderly patients: a randomized controlled preliminary trial[J]. Chin Med J, 2019, 132(4): 437-445. DOI: 10.1097/CM9.0000000000000098.

[12] ZOU L L, NING M L, WANG W J, *et al*. Naringenin prevents propofol induced neurodegeneration in neonatal mice brain and long-term neurocognitive impacts on adults [J]. Drug Des Devel Ther, 2020, 14: 5469-5482. DOI: 10.2147/DDDT.S280443.

[13] XING N, XING F, LI Y N, *et al*. Dexmedetomidine improves propofol-induced neuronal injury in rat hippocampus with the involvement of miR-34a and the PI3K/Akt signaling pathway [J]. Life Sci, 2020, 247: 117359. DOI: 10.1016/j.lfs.2020.117359.

[14] 于晴, 刘阳, 孙茫, 等. 低氧因素对丙泊酚麻醉新生大鼠认知功能的影响[J]. 中国临床药理学杂志, 2018, 34(14): 1678-1681. DOI: 10.13699/j.cnki.1001-6821.2018.14.027.

YU Q, LIU Y, SUN M, *et al*. Effect of hypoxic factors on cognitive function in propofol-anesthetized neonatal rats[J]. Chin J Clin Pharmacol, 2018, 34(14): 1678-1681. DOI: 10.13699/j.cnki.1001-6821.2018.14.027.

[15] 高雯, 王维萍, 刘玲, 等. 低氧复合丙泊酚通过铁死亡对未成熟SD大鼠海马神经元的影响[J]. 陆军军医大学学报, 2023, 45(5): 380-385. DOI: 10.16016/j.2097-0927.202208021.

GAO W, WANG W P, LIU L, *et al*. Hypoxia aggravates propofol-induced hippocampal neuron injury in immature SD rats through ferroptosis [J]. J Army Med Univ, 2023, 45(5): 380-385. DOI: 10.16016/j.2097-0927.202208021.

[16] 张敬, 于晴, 刘阳, 等. 丙泊酚合并低氧通过p38通路损伤未成熟大鼠的认知功能[J]. 南方医科大学学报, 2018, 38(11): 1294-1299. DOI: 10.12122/j.issn.1673-4254.2018.11.03.

ZHANG J, YU Q, LIU Y, *et al*. Propofol combined with hypoxia induces cognitive dysfunction in immature rats *via* p38 pathway[J]. J South Med Univ, 2018, 38(11): 1294-1299. DOI: 10.12122/j.issn.1673-4254.2018.11.03.

[17] PALIKARAS K, LIONAKI E, TAVERNARAKIS N. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology[J]. Nat Cell Biol, 2018, 20(9): 1013-1022. DOI: 10.1038/s41556-018-0176-2.

[18] LAZAROU M, SLITER D A, KANE L A, *et al*. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy[J]. Nature, 2015, 524(7565): 309-314. DOI: 10.1038/nature14893.

[19] GEORGAKOPOULOS N D, WELLS G, CAMPANELLA M. The pharmacological regulation of cellular mitophagy [J]. Nat Chem Biol, 2017, 13(2): 136-146. DOI: 10.1038/nchembio.2287.

[20] YAPA N M B, LISNYAK V, RELJIC B, *et al*. Mitochondrial dynamics in health and disease[J]. FEBS Lett, 2021, 595(8): 1184-1204. DOI: 10.1002/1873-3468.14077.

[21] LIANG Y B, HUANG Y, SHAO R G, *et al*. Propofol produces neurotoxicity by inducing mitochondrial apoptosis[J]. Exp Ther Med, 2022, 24(4): 630. DOI: 10.3892/etm.2022.11567.

[22] GUO P, MEI J. Exploring the correlation between Drp1 protein and the neurotoxicity of propofol[J]. Front Neurosci, 2025, 19: 1614362. DOI: 10.3389/fnins.2025.1614362.

[23] CASSIDY-STONE A, CHIPUK J E, INGERMAN E, *et al*. Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization[J]. Dev Cell, 2008, 14(2): 193-204. DOI: 10.1016/j.devcel.2007.11.019.

[24] SMITH G, GALLO G. To mdivi-1 or not to mdivi-1: Is that the question[J]. Dev Neurobiol, 2017, 77(11): 1260-1268. DOI: 10.1002/dneu.22519.

[25] OLIVER D, REDDY P H. Dynamics of dynamin-related protein 1 in Alzheimer's disease and other neurodegenerative diseases[J]. Cells, 2019, 8(9): 961. DOI: 10.3390/cells8090961.

[26] ONG S B, KWEK X Y, KATWADI K, *et al*. Targeting mitochondrial fission using mdivi-1 in a clinically relevant large animal model of acute myocardial infarction: a pilot study[J]. Int J Mol Sci, 2019, 20(16): 3972. DOI: 10.3390/ijms20163972.

[27] YANG M J, LINN B S, ZHANG Y M, *et al*. Mitophagy and mitochondrial integrity in cardiac ischemia-reperfusion injury[J]. Biochim Biophys Acta Mol Basis Dis, 2019, 1865(9): 2293-2302. DOI: 10.1016/j.bbadis.2019.05.007.

[28] 李艳花, 张晓娟, 张思羽, 等. Mdivi-1通过抑制少突胶质细胞凋亡信号通路发挥髓鞘保护作用[J]. 中国病理生理杂志, 2024, 40(3): 527-534. DOI: 10.3969/j.issn.1000-4718.2024.00.011.
LI Y H, ZHANG X J, ZHANG S Y, *et al*. Mdivi-1 protects oligodendrocytes through inhibiting apoptotic signaling pathway[J]. Chin J Pathophysiol, 2024, 40(3): 527-534. DOI: 10.3969/j.issn.1000-4718.2024.00.011.

[29] 铁静静, 李晓东, 倪子薇, 等. Mdivi-1通过修复黑质网状部线粒体改善肝性脑病小鼠运动功能[J]. 神经解剖学杂志, 2024, 40(4): 413-420. DOI: 10.16557/j.cnki.1000-7547.2024.04.002.
TIE J J, LI X D, NI Z W, *et al*. Mdivi-1 improves motor function of mice with hepatic encephalopathy by repairing mitochondrion in substantia nigra Reticulata[J]. Chin J Neuroanat, 2024, 40(4): 413-420. DOI: 10.16557/j.cnki.1000-7547.2024.04.002.

[30] 汤春琼, 朱娱, 王易, 等. 线粒体分裂抑制剂Mdivi-1对脓毒症大鼠肠道屏障功能的保护作用[J]. 陆军军医大学学报, 2024, 46(5): 419-426. DOI: 10.16016/j.2097-0927.202303166.
TANG C Q, ZHU Y, WANG Y, *et al*. Mitochondrial division inhibitor Mdivi-1 protects intestinal barrier function in septic rats[J]. J Army Med Univ, 2024, 46(5): 419-426. DOI: 10.16016/j.2097-0927.202303166.

[31] CHUANG Y C, LIN T K, YANG D I, *et al*. Peroxisome proliferator-activated receptor-gamma dependent pathway reduces the phosphorylation of dynamin-related protein 1 and ameliorates hippocampal injury induced by global ischemia in rats[J]. J Biomed Sci, 2016, 23(1): 44. DOI: 10.1186/s12929-016-0262-3.

[32] 胡锦涛, 焦晶晶, 彭晓红. Mdivi-1减轻异丙酚诱导的发育期神经元凋亡[J]. 中国医院药学杂志, 2019, 39(1): 53-56, 71. DOI: 10.13286/j.cnki.chinhosppharmacyj.2019.01.11.
HU J T, JIAO J J, PENG X H. Mdivi-1 mitigates propofol-induced developmental hippocampal neuroapoptosis *in vitro*[J]. Chin J Hosp Pharm, 2019, 39(1): 53-56, 71. DOI: 10.13286/j.cnki.chinhosppharmacyj.2019.01.11.

[33] LI W L, JI M L, LIN Y D, *et al*. DEPP/DEPP1/C10ORF10 regulates hepatic glucose and fat metabolism partly *via* ROS-induced FGF21[J]. FASEB J, 2018, 32(10): 5459-5469. DOI: 10.1096/fj.201800357r.

[34] WU Q, GAO C, WANG H C, *et al*. Mdivi-1 alleviates blood-brain barrier disruption and cell death in experimental traumatic brain injury by mitigating autophagy dysfunction and mitophagy activation[J]. Int J Biochem Cell Biol, 2018, 94: 44-55. DOI: 10.1016/j.biocel.2017.11.007.

[35] MARX N, RITTER N, DISSE P, *et al*. Detailed analysis of Mdivi-1 effects on complex I and respiratory super complex assembly[J]. Sci Rep, 2024, 14(1): 19673. DOI: 10.1038/s41598-024-69748-y.

(**收稿**:2025-05-29;**修回**:2025-10-27)
(编辑 严 涵)