

TUG-891 对缺血缺氧诱导的缺血性脑卒中的保护作用及其机制

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[摘 要] **目的:** 探讨 TUG-891 对缺血缺氧诱导的缺血性脑卒中 (IS) 的保护作用, 并阐明其可能的作用机制。**方法:** 将 60 只健康雄性 C57BL/6 小鼠随机分为假手术组、模型组 [远端大脑中动脉闭塞 (dMCAO) 组] 和 dMCAO+TUG-891 组 (dMCAO+TUG-891 组), 每组 20 只, 造模后连续 3 d 给予小鼠腹腔注射 TUG-891 溶液 ($35 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$)。采用改良神经功能缺损评分 (mNSS) 和转棒实验评估各组小鼠神经功能情况, 2, 3, 5-氯化三苯基四氮唑 (TTC) 染色法观察各组小鼠脑梗死体积, 生化法检测各组小鼠脑组织上清液中丙二醛 (MDA) 水平和超氧化物歧化酶 (SOD) 活性, 苏木精-伊红 (HE) 和尼氏 (NISSL) 染色观察各组小鼠脑组织病理形态表现, 末端脱氧核苷酸转移酶 dUTP 缺口末端标记法 (TUNEL) 染色检测各组小鼠脑组织中神经细胞凋亡指数, Western blotting 法检测各组小鼠脑组织中 G 蛋白偶联受体 78 (GPR78)、PKR 样内质网激酶 (PERK) 和磷酸化 PERK (p-PERK) 和 CHOP 蛋白表达水平。**结果:** mNSS 和转棒实验, 与假手术组比较, dMCAO 组小鼠 mNSS 明显升高 ($P < 0.01$), 在棒时间明显减少 ($P < 0.01$); 与 dMCAO 组比较, dMCAO+TUG-891 组小鼠 mNSS 降低 ($P < 0.05$), 在棒时间增加 ($P < 0.05$)。TTC 染色法, 与假手术组比较, dMCAO 组小鼠脑梗死体积增大 ($P < 0.01$); 与 dMCAO 组比较, dMCAO+TUG-891 组小鼠脑梗死体积明显减小 ($P < 0.01$)。HE 染色, 与假手术组比较, dMCAO 组小鼠大脑皮层严重受损, 表现为梗死区神经细胞排列紊乱及细胞核明显固缩。dMCAO+TUG-891 组小鼠脑梗死区神经细胞形态损伤得到明显改善。NISSL 染色, dMCAO 组小鼠大脑皮层梗死区尼氏小体变细拉长, 脱失增多; dMCAO+TUG-891 组小鼠脑组织病理损伤均得到明显改善。与假手术组比较, dMCAO 组大鼠脑组织中 MDA 水平明显升高 ($P < 0.01$), SOD 活性降低 ($P < 0.01$); 与 dMCAO 组比较, dMCAO+TUG-891 组小鼠脑组织中 MDA 水平明显降低 ($P < 0.01$), SOD 活性明显升高 ($P < 0.01$)。TUNEL 染色, 与假手术组比较, dMCAO 组小鼠脑组织中神经细胞凋亡指数升高 ($P < 0.01$); 与 dMCAO 组比较, dMCAO+TUG-891 组小鼠脑组织中神经细胞凋亡指数降低 ($P < 0.01$)。与假手术组比较, dMCAO 组小鼠脑组织中 GRP78、p-PERK 和 CHOP 蛋白表达水平升高 ($P < 0.05$); 与 dMCAO 组比较, dMCAO+TUG-891 组小鼠脑组织中 GRP78、p-PERK 和 CHOP 蛋白表达水平降低 ($P < 0.05$)。**结论:** TUG-891 可以减轻 IS 引起的神经损伤, 其作用机制可能与 TUG-891 抑制内质网应激和细胞凋亡有关。

[关键词] 缺血性脑卒中; G 蛋白偶联受体 78; TUG-891; 内质网应激; 细胞凋亡

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Protective effect of TUG-891 on ischemic stroke induced by ischemia and hypoxia and its mechanism

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ABSTRACT Objective: To discuss the protective effect of TUG-891 on ischemic stroke (IS) induced by ischemia-hypoxia, and to clarify its potential mechanism. **Methods:** A total of 60 healthy male C57BL/6 mice were randomly divided into sham operation group ($n=20$), model group [distal middle cerebral artery occlusion (dMCAO) group, $n=20$], and model+TUG-891 group (dMCAO+TUG-891 group, $n=20$). After modeling, the mice were intraperitoneally injected with TUG-891 solution ($35 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) for 3 consecutive days. Modified neurological severity score (mNSS) and rotarod test were used to evaluate the neurological function of the mice in various groups; 2,3,5-triphenyltetrazolium chloride (TTC) staining was used to observe the cerebral infarction volumes of the mice in various groups; biochemical method was used to detect the malondialdehyde (MDA) level and superoxide dismutase (SOD) activity in the supernatant of brain tissue of the mice in various groups; Hematoxylin-Eosin (HE) and NISSL staining were used to observe the pathomorphology of brain tissue of the mice in various groups; terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining was used to detect the apoptotic indexes of neuronal cells in brain tissue of the mice in various groups; Western blotting method was used to detect the expression levels of glucose-regulated protein 78 (GRP78), protein kinase R-like endoplasmic reticulum kinase (PERK), phosphorylated PERK (p-PERK), and C/EBP homologous protein (CHOP) proteins in brain tissue of the mice in various groups. **Results:** The mNSS and rotarod test results showed that compared with sham operation group, the mNSS of the mice in dMCAO group was significantly increased ($P<0.01$), and the time on the rod was significantly decreased ($P<0.01$); compared with dMCAO group, the mNSS of the mice in dMCAO+TUG-891 group was decreased ($P<0.05$), and the time on the rod was increased ($P<0.05$). The TTC staining results showed that compared with sham operation group, the volume of white infarct foci in the cerebral cortex of the mice in dMCAO group was increased ($P<0.01$); compared with dMCAO group, the cerebral infarction volume of the mice in dMCAO+TUG-891 group was significantly decreased ($P<0.01$). The HE staining results showed that compared with sham operation group, the cortex of the mice in dMCAO group was severely damaged, manifested by disordered arrangement of neuronal cells and obvious nuclear pyknosis in the infarct area, and the morphology of cortical infarct area of the mice in dMCAO+TUG-891 group was improved; the NISSL staining results showed that the Nissl bodies in the cortical infarct area of the mice in dMCAO group became thinner, elongated, and lost more. The pathological damage of brain tissue of the mice in dMCAO+TUG-891 group was significantly improved. Compared with sham operation group, the MDA level in brain tissue of the mice in model group was significantly increased ($P<0.01$), and the SOD activity was decreased ($P<0.01$); compared with model group, the MDA level in brain tissue of the mice in TUG-891 group was significantly decreased ($P<0.01$), and the SOD activity was significantly increased ($P<0.01$). The TUNEL staining results showed that compared with sham operation group, the apoptotic index of neuronal cells in brain tissue of the mice in dMCAO group was increased ($P<0.01$); compared with dMCAO group, the apoptotic index of neuronal cells in brain tissue of the mice in dMCAO+TUG-891 group was decreased ($P<0.01$). Compared with sham operation group, the expression levels of GRP78, p-PERK, and CHOP proteins in brain tissue of the mice in dMCAO group were increased ($P<0.05$); compared with

dMCAO group, the expression levels of GRP78, p-PERK, and CHOP proteins in brain tissue of the mice in dMCAO+TUG-891 group were decreased ($P<0.05$). **Conclusion:** TUG-891 can alleviate neurological injury caused by ischemic stroke, and its mechanism may be related to the inhibition of endoplasmic reticulum stress and apoptosis.

KEYWORDS Ischemic stroke; G protein-coupled receptor 78; TUG-891; Endoplasmic reticulum stress; Apoptosis

缺血性脑卒中(ischemic stroke, IS)是由于脑动脉狭窄或闭塞引起脑供血不足进而诱导脑组织损伤,并表现出相应神经功能缺损的一种脑血管疾病,其发生率超过80%^[1]。目前临床上治疗主要是通过超急性期使用组织型纤溶酶原激活剂(tissue plasminogen activator, tPA)或者急诊手术的方式实现血管再灌注^[2]。但由于血管再灌注治疗时间窗窄,限制了其应用^[3],因此急需寻找新的治疗途径。大脑中富含必需的 ω -3多不饱和脂肪酸,通过激活G蛋白偶联受体120(Gprotein-coupled receptor 120, GPR120)发挥神经保护作用^[4];但是 ω -3多不饱和脂肪酸对GPR120的激动作用持续疗效较低且特异性不强;此外, ω -3多不饱和脂肪酸在细胞内代谢后通过代谢物调节细胞功能,严重干扰了对实验结果准确性和可靠性的解释^[5-6]。基于以上缺点, ω -3多不饱和脂肪酸未被临床上广泛应用,而TUG-891的出现弥补了上述缺陷^[7]。研究^[8]显示:TUG-891对GPR120受体的选择性是其他多不饱和脂肪酸受体的1000倍,而且对实验动物相对安全。TUG-891是一种新型选择合成的GPR120的激动剂,其不会在细胞内代谢,而是通过与配体直接结合后将其激活,在改善炎症,增强胰岛素敏感性和改变脂质代谢等方面发挥作用^[9-10]。在动物实验中,TUG-891已被证实对多种神经系统疾病具有一定的治疗潜力,其能够改善认知功能障碍和记忆损伤,表现出明显的神经保护作用^[11]。在急性肾损伤疾病模型中,TUG-891也被证实可以抑制内质网应激,减少顺铂引起的肾脏伤害^[12]。但TUG-891对缺血性脑损伤的作用目前尚未见相关报道。本研究建立小鼠远端大脑中动脉闭塞(distal middle cerebral artery occlusion, dMCAO)模型,探讨TUG-891是否对缺血缺氧诱导的IS具有保护作用及其可能的作用机制,为TUG-891的临床开发和应用提供理论依据。

1 材料与方法

1.1 实验动物、主要试剂和仪器 60只正常雄性

C57BL/6小鼠,体质量20~25 g,8~10周龄,由北京大学医学部动物部提供,动物使用许可证号:SYXK(京)2022-0037。小鼠饲养在温度23℃±2℃,湿度60%±5%的环境中,并给予光照12 h/黑暗12 h交替,所有小鼠提供标准饮食水。TUG-891(美国MCE公司),2,3,5-氯化三苯基四氮唑(2,3,5-triphenyltetrazole chloride, TTC)染色试剂粉末、苏木精-伊红(hematoxylin-eosin, HE)染色试剂盒和尼氏(NISSL)染色试剂盒(北京索莱宝生物科技有限公司),末端脱氧核苷酸转移酶dUTP缺口末端标记法(terminal deoxynucleotidyl transferase dUTP nick-end labeling, TUNEL)凋亡检测试剂盒(武汉普诺赛生命科技有限公司),丙二醛(malondialdehyde, MDA)、超氧化物歧化酶(superoxide dismutase, SOD)、G蛋白偶联受体78(G protein-coupled receptor 78, GPR78)和CHOP(武汉三鹰生物技术有限公司),PKR样内质网激酶(PKR-like endoplasmic reticulum kinase, PERK)、磷酸化PERK(phosphorylated PERK, p-PERK)(上海Abmart公司), β -actin(北京Signalway Antibody公司),蛋白酶、磷酸酶抑制剂和BCA蛋白检测试剂盒(北京普利莱基因技术有限公司)。Western blotting系列仪器(北京六一生物科技有限公司),数字切片扫描仪和颅骨钻(天津赛德生物制药有限公司),电凝笔(上海鑫工生物医药有限公司)。

1.2 实验动物模型制备、分组和给药 将小鼠进行永久性大脑中动脉闭塞,具体操作步骤:小鼠体温维持在37℃±0.5℃,采用2.5%三溴乙醇(0.16 mL/10 g)诱导麻醉后,对其右侧颈总动脉进行永久性结扎并缝合伤口。在小鼠右侧眼外眦和右侧外耳道之间作1个长度为0.5 cm的切口,剪开颞肌并暴露颞骨,在显微镜下可见右内侧大脑皮层的颅下分支,用颅骨钻在颅骨上钻1个直径约2 mm的小孔,以暴露右侧大脑中动脉的皮质分支,然后用电凝笔将动脉灼烧中断。假手术组小鼠右颈总动脉分离后不进行结扎,钻孔暴露大脑中动脉后

不烧灼。将小鼠随机分为假手术组、dMCAO组和dMCAO+TUG-891组,每组20只。TUG-891按照说明书进行溶解,于造模后1、24和48 h分别给予dMCAO+TUG-891组小鼠腹腔注射。假手术组和dMCAO组小鼠给予等体积二甲基亚砜(dimethyl sulfoxide, DMSO)溶液。

1.3 改良神经功能缺损评分(modified neurological severity score, mNSS)评估各组小鼠神经功能 为检测小鼠在脑缺血后的神经功能损伤程度,在安静的环境中采用双盲法对小鼠进行mNSS测定。mNSS主要用于评估小鼠的运动、感觉、平衡和反射,量表分数为0~18分。正式实验时,在相对安静的环境中进行mNSS行为测试,并记录每组数据。0分为最低分,18分为最高分,得分越高表明小鼠神经功能状态越差。

1.4 转棒实验检测各组小鼠运动能力 每组取8只小鼠,转棒测试用于评估啮齿类动物的运动协调和平衡能力。正式实验前需要先进行预训练以尽量减少可能导致小鼠焦虑和探索行为的潜在因素。在实验开始前4 d,以 $4\text{ r}\cdot\text{min}^{-1}$ 转速对小鼠进行训练实验,每天3次,每次5 min。训练期间,如果小鼠从旋转杆上掉下来,可将小鼠放回杆上继续训练。正式实验时将小鼠置于旋转杆上,并在5 min内将杆的转速从 $4\text{ r}\cdot\text{min}^{-1}$ 增加到 $40\text{ r}\cdot\text{min}^{-1}$,记录小鼠在棒时间,每组检验3次。小鼠在棒时间越长,表明其神经损伤越小。

1.5 TTC染色检测各组小鼠脑梗死体积 造模72 h后,各组取5只小鼠,采用三溴乙醇麻醉并迅速断头取脑,置于 $-20\text{ }^{\circ}\text{C}$ 冷冻20 min,将脑组织切成厚度为2 mm冠状面并置于2%的TTC染液中避光染色30 min。采用Image J软件计算各组小鼠脑组织梗死体积。正常脑组织为红色,梗死区为白色,白色区域越大代表小鼠脑梗死越大。计算公式:脑梗死率=(梗死面积/全脑面积) $\times 100\%$ 。以脑梗死率代表脑梗死体积。

1.6 HE染色和NISSL染色观察各组小鼠脑组织病理形态表现 对小鼠进行深度麻醉,从左心室分别用生理盐水和4%多聚甲醛进行灌注,并进一步固定、脱水、石蜡包埋和切片后行HE和NISSL染色。HE染色和NISSL染色根据染色试剂盒说明书进行操作。在显微镜下观察皮质梗死和半暗带区神经细胞及尼氏小体的变化。神经元和Nissl小体形态越好代表神经细胞损伤越小。

1.7 生化试剂盒检测各组小鼠脑组织中MDA水平和SOD活性 将小鼠脑组织在冷磷酸盐缓冲液(phosphated buffer saline, PBS)中匀浆,12 000 g离心10 min收集上清液,然后根据生化试剂盒说明书进行MDA水平和SOD活性检测。MDA水平越高和SOD活性越低表明小鼠脑组织氧化损伤程度越严重。

1.8 TUNEL染色检测各组小鼠脑组织细胞凋亡指数 取出提前制备好的小鼠脑组织石蜡切片,严格按照TUNEL原位细胞凋亡检测试剂盒说明书进行染色,染色完成后采用荧光显微镜观察细胞凋亡情况,最后通过Image J软件分析细胞凋亡情况。荧光信号越强,染色数越多,则代表细胞凋亡率越高。细胞凋亡指数=荧光图片总吸光度(A)值/各组细胞对应总面积。

1.9 Western blotting法检测各组小鼠脑组织中氧化应激相关蛋白表达水平 将行为学实验结束后的各组小鼠麻醉致死并取梗死组织,于 $-80\text{ }^{\circ}\text{C}$ 冰箱保存。将小鼠脑组织快速匀浆提取蛋白,采用BCA蛋白浓度测定法定量蛋白后进行电泳、转膜、封闭,在一抗、二抗孵育后使用发光液显影,并采用Image J软件对各蛋白条带灰度值进行计算以量化目的蛋白表达水平。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Graphpad Prism 9.0.0软件进行统计学分析。各组小鼠mNSS,在棒时间,脑梗死体积,脑组织中MDA水平和SOD活性,细胞凋亡指数及脑组织中CHOP、GRP78、PERK和p-PERK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,组间样本均数两两比较采用LSD-*t*检验。以 $P<0.05$ 为差异有统计学意义。

2 结果

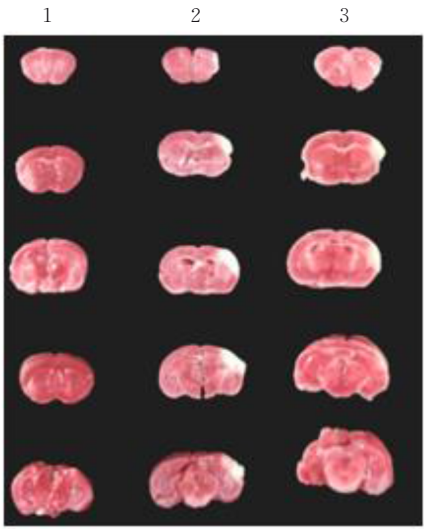
2.1 各组小鼠mNSS和在棒时间 与假手术组比较,dMCAO组小鼠mNSS明显升高($P<0.01$),在棒时间明显减少($P<0.01$),表明小鼠神经功能受损明显;与dMCAO组比较,dMCAO+TUG-891组小鼠mNSS降低($P<0.05$),在棒时间增加($P<0.05$)。见表1。

2.2 各组小鼠脑梗死体积 与假手术组比较,dMCAO组小鼠脑梗死体积增大($P<0.01$);与dMCAO组比较,dMCAO+TUG-891组小鼠脑梗死体积明显减小($P<0.01$)。见图1和2。

表 1 各组小鼠 mNSS 和在棒时间
Tab. 1 mNSS and beam walking time of mice in various groups
($n=8, \bar{x} \pm s$)

Group	mNSS	Beam walking time (t/s)
Sham operation	0.24±0.10	289.00±23.93
dMCAO	13.37±0.82*	163.00±30.05*
dMCAO+TUG-891	9.88±0.53 [△]	208.00±19.27 [△]

* $P<0.01$ vs sham operation group; [△] $P<0.01$ vs dMCAO group.

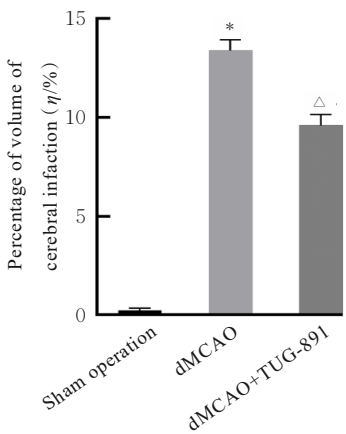


Lane 1: Sham operation group; Lane 2: dMCAO group;
Lane 3: dMCAO+TUG-891 group.

图 1 各组小鼠脑梗死形态表现

Fig. 1 Morphology of cerebral infarction of mice in various groups

2.3 各组小鼠脑组织病理形态表现 HE 染色结果显示：与假手术组比较，dMCAO 组小鼠大脑皮层严重受损，表现为梗死区神经细胞排列紊乱及细胞核明显固缩；dMCAO+TUG-891 组小鼠梗死区神



* $P<0.01$ vs sham operation group; [△] $P<0.01$ vs dMCAO group.

图 2 各组小鼠脑梗死体积

Fig. 2 Volume of cerebral infarction of mice in various groups

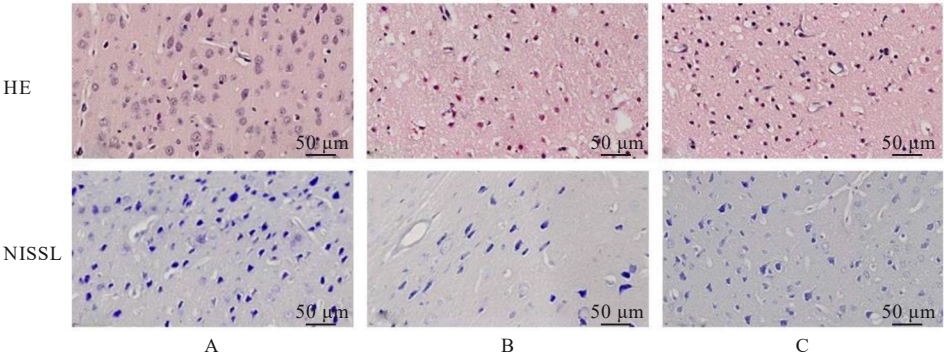
经细胞形态有所改善。NISSL 染色结果显示：dMCAO 组小鼠大脑皮层梗死区尼氏小体变细拉长，脱失增多。dMCAO+TUG-891 组小鼠脑组织病理损伤均得到明显改善。见图 3。

2.4 各组小鼠脑组织中 MDA 水平和 SOD 活性

与假手术组比较，dMCAO 组小鼠脑组织中 MDA 水平明显升高 ($P<0.01$)，SOD 活性降低 ($P<0.01$)；与 dMCAO 组比较，dMCAO+TUG-891 组小鼠脑组织中 MDA 水平明显降低 ($P<0.01$)，SOD 活性升高 ($P<0.01$)。见表 2。

2.5 各组小鼠脑组织中神经细胞凋亡指数

TUNEL 染色结果显示：与假手术组比较，dMCAO 组小鼠脑组织中神经细胞凋亡指数升高 ($P<0.01$)；与 dMCAO 组比较，dMCAO+TUG-891 组小鼠脑组织中神经细胞凋亡指数降低 ($P<0.01$)。见图 4 和 5。



A: Sham operation group; B: dMCAO group; C: dMCAO+TUG-891 group.

图 3 各组小鼠脑组织病理形态表现

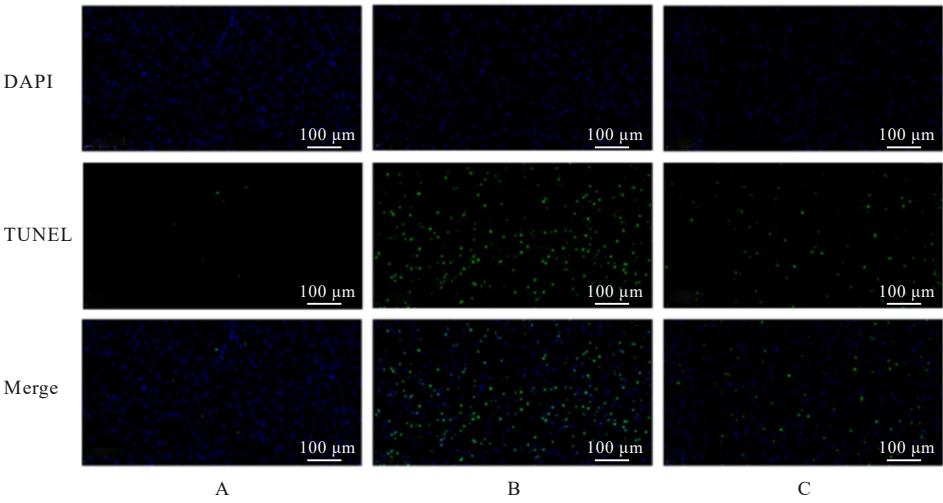
Fig. 3 Pathomorphology of of brain tissue of mice in various groups

表2 各组小鼠脑组织中MDA水平和SOD活性
Tab. 2 Levels of MDA and activities of SOD in brain tissue of mice in various groups (n=5, $\bar{x}\pm s$)

Group	MDA [$m_B/(\mu\text{mol}\cdot\text{g}^{-1})$]	SOD [$\lambda_B/(\text{U}\cdot\text{mg}^{-1})$]
Sham operation	5.20±0.20	76.47±3.80
dMCAO	7.31±0.13*	48.40±4.84*
dMCAO+TUG-891	5.99±0.14 [△]	63.73±4.08 [△]

*P<0.01 vs sham operation group; [△]P<0.01 vs dMCAO group.

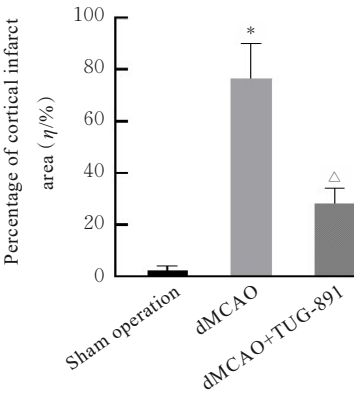
2.6 各组小鼠脑组织中内质网应激相关蛋白表达水平 与假手术组比较, dMCAO组小鼠脑组织中CHOP、GRP78和p-PERK蛋白表达水平升高(P<0.05); 与dMCAO组比较, dMCAO+TUG-891组小鼠脑组织中CHOP、p-PERK和GRP78蛋白表达水平降低(P<0.05)。见图6。



A: Sham operation group; B: dMCAO group; C: dMCAO+TUG-891 group.

图4 各组小鼠脑组织中神经细胞凋亡形态表现 (TUNEL)

Fig. 4 Apoptotic morphology of neurons in brain tissue of mice in various groups (TUNEL)



*P<0.01 vs sham operation group; [△]P<0.01 vs dMCAO group.

图5 各组小鼠脑组织中神经细胞凋亡指数

Fig. 5 Apoptotic indexes of neurons in brain tissue of mice in various groups

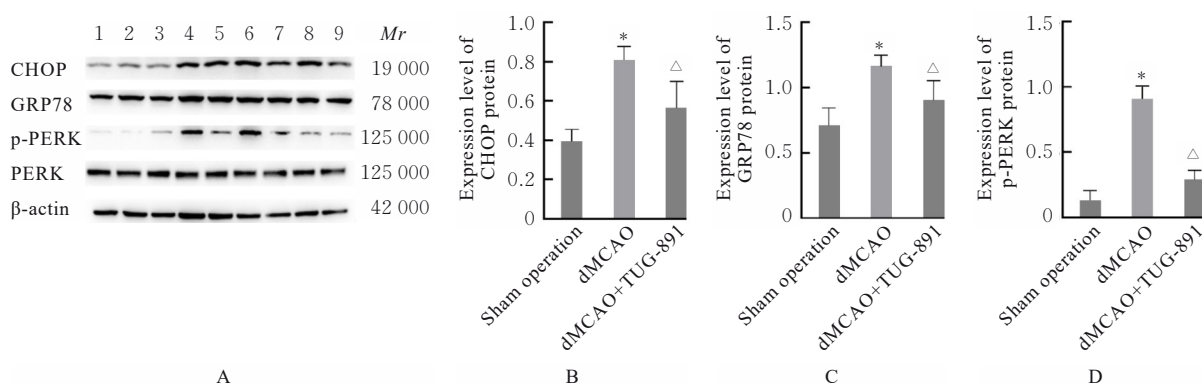
3 讨论

为了证实 TUG-891对缺血缺氧诱导的脑组织损伤是否有保护作用, 通过建立大脑中动脉远端闭塞模型来模拟缺血性脑损伤的改变^[13]。本研究结

果显示: dMCAO小鼠经过TUG-891处理后脑梗死体积明显减小, 通过mNSS评分和转棒实验证实了给予TUG-891治疗可明显改善小鼠神经功能损伤; HE染色和NISSL染色结果显示: 与dMCAO组比较, dMCAO+TUG-891组小鼠脑组织中神经细胞丢失以及形态和结构改变均有所减少或减轻; 表明TUG-891在缺血性脑损伤中的神经保护作用。

氧化应激、内质网应激和凋亡在脑缺血损伤中的病理生理机制已被广泛证实^[14-17]。因此, 调节氧化应激水平、细胞凋亡和内质网应激信号可能是预防IS的一种有希望的治疗方法。抗氧化剂SOD的消耗和MDA的积累直接反映了IS的脂质过氧化程度。本研究通过检测各组小鼠脑组织中MDA水平和SOD活性, 观察到TUG-891对脑缺血过程中引起的氧化应激有一定影响, 结果表明: IS启动氧化应激激活, 产生过量MDA并降低SOD活性, 但上述变化均被TUG-891所抑制。

研究^[18]显示: 氧化应激多伴随内质网功能障碍。脑缺血后引起的内质网应激可能是导致缺血期



A: Electrophoregram (Lane 1-3: Sham operation group; Lane 4-6: dMCAO group; Lane 7-9: dMCAO+TUG-891 group); B-D: Histograms; B: CHOP; C: GRP78; D: p-PERK. * $P < 0.05$ vs sham operation group; $\Delta P < 0.05$ vs dMCAO group.

图6 各组小鼠脑组织中CHOP、GRP78和p-PERK蛋白表达电泳图(A)及直条图(B~D)

Fig. 6 Electrophoregram (A) and histograms (B-D) of expressions of CHOP, GRP78, and p-PERK proteins in brain tissue of mice in various groups

间或恢复期神经功能障碍的主要复杂病理机制之一,也是脑缺血损伤干预的潜在靶点。内质网发生应激反应可激活多种信号通路去除未折叠或错误折叠蛋白,重新平衡细胞间的稳定状态,被称为未折叠蛋白反应。未折叠蛋白反应作为压力传感器和下游转录因子的调节器监测未折叠及错误折叠的蛋白质聚集并重新编码基因^[19],其特点是激活3个主要传感器:PERK、肌醇需求酶1(inositol-requiring enzyme 1, IRE1)和激活转录因子6(activating transcription factor 6, ATF6)。生理条件下,上述3种传感器通过与GRP78结合而受到抑制,但是当内质网应激时,关键的跨膜蛋白PERK与GRP78分离并发生磷酸化激活下游通路,最终诱导细胞凋亡^[20]。因此靶向抑制内质网应激和UPR可有效改善缺血后脑损伤。研究^[21]显示:黄芪甲苷类黄酮通过抑制短暂性大脑中动脉闭塞后内质网应激,明显降低内质网相关蛋白的表达而减轻缺血性脑损伤。研究^[22]显示:毛状样及分裂增强子1(hairy and enhancer of split 1, *Hes1*)基因下调可通过PERK途径加重神经细胞凋亡,其机制可能是由于PERK特异性抑制剂GSK2606414可以抑制梗死体积的增加和神经系统的损伤。本研究结果显示:TUG-891通过降低PERK磷酸化和GRP78水平来抑制PERK通路的激活,表明TUG-891可以逆转内质网应激。

PERK的激活诱导CHOP表达。CHOP是内质网应激诱导细胞凋亡的指标之一,在生理条件下处于低水平,在组织或细胞受到应激刺激时明显升

高,但其不能直接引起细胞凋亡,而是通过调节下游凋亡相关因子的表达介导细胞凋亡^[23]。LI等^[22]发现:PERK-CHOP的激活加重了IS,而Hes1通过阻止PERK-CHOP通路诱导的细胞凋亡发挥保护作用。本研究结果显示:脑缺血后使用TUG-891治疗可明显抑制IS诱导的CHOP表达;TUNEL染色结果显示:TUG-891可以减轻IS后由内质网应激所诱导的细胞凋亡,表明TUG-891可以通过PERK-CHOP轴减弱内质网应激及其诱导的细胞凋亡从而拮抗IS诱导的缺血性损伤。

综上所述,TUG-891对缺血引起的脑损伤具有明显的保护作用,其保护作用可能是通过抑制内质网应激和细胞凋亡来实现的,TUG-891可能是一种治疗IS的新型潜在临床药物。

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作者贡献声明:

孙攀喜参与实验方案设计、实验操作和论文撰写,秦雪参与动物模型制备和取材,张重阳和罗佳参与实验设计及数据收集,陈勇和魏丽丽参与实验设计指导及论文审校。

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