DOI：10.16781/j.CN31-2187/R.20220615

·论　著·

替罗非班通过 SIRT1/VEGF 信号通路减轻急性脑梗死大鼠神经元损伤

王文文[1]，邵彦江[2]，马　琪[1]，张新乐[1]，杨改清[3]，徐国卫[3*]
1. 郑州市第七人民医院药学部，郑州 450016
2. 郑州大学药学院药物分析教研室，郑州 450000
3. 郑州大学附属郑州中心医院神经内科，郑州 450001

［摘要］ **目的**　探讨替罗非班能否通过沉默信息调节因子 2 相关酶 1（SIRT1）/ 血管内皮生长因子（VEGF）信号通路减轻急性脑梗死（ACI）大鼠神经元损伤。**方法**　将 75 只 SD 大鼠随机分为假手术组、模型组、替罗非班（60 μg/kg）组、SIRT1 抑制剂（5 mg/kg SIRT1 特异性抑制剂 EX-527）组、替罗非班＋SIRT1 抑制剂组，每组 15 只。除假手术组外，其他 4 组大鼠构建 ACI 模型。对各组大鼠进行神经功能评分；采用氯化三苯基四氮唑染色检测大鼠脑梗死体积百分数；采用硫代巴比妥酸法检测大鼠血清丙二醛水平，采用比色法检测血清谷胱甘肽过氧化物酶（GSH-Px）水平，采用微板法检测血清超氧化物歧化酶（SOD）水平；采用 H-E 染色检测大鼠脑组织病理变化；采用 TUNEL 染色检测大鼠神经元凋亡水平；采用蛋白质印迹法检测大鼠海马组织中 SIRT1、VEGF 蛋白的表达。**结果**　与假手术组比较，模型组大鼠脑组织海马区病理损伤严重，神经功能评分、脑梗死体积百分数、血清丙二醛水平、神经元凋亡率均较高（P 均＜0.05），血清 GSH-Px、SOD 水平及海马组织中 SIRT1、VEGF 蛋白表达水平均降低（P 均＜0.05）。与模型组比较，替罗非班组、替罗非班＋SIRT1 抑制剂组大鼠脑组织海马区病理损伤减轻，神经功能评分、脑梗死体积百分数、血清丙二醛水平、神经元凋亡率均降低（P 均＜0.05），血清 GSH-Px、SOD 水平及海马组织中 SIRT1、VEGF 蛋白表达水平均升高（P 均＜0.05）；而 SIRT1 抑制剂组大鼠相应指标变化呈相反趋势（P 均＜0.05）。**结论**　替罗非班可能通过激活 SIRT1/VEGF 信号通路抑制氧化应激和神经元凋亡，进而减轻 ACI 大鼠的神经元损伤。

［关键词］ 替罗非班；急性脑梗死；沉默信息调节因子 2 相关酶 1；血管内皮生长因子；神经元损伤；细胞凋亡；氧化性应激
［中图分类号］ R 743.3　　［文献标志码］ A　　［文章编号］ 2097-1338(2023)06-0672-07

Tirofiban alleviating neuronal injury in acute cerebral infarction rats by regulating SIRT1/VEGF signaling pathway

WANG Wen-wen[1], SHAO Yan-jiang[2], MA Qi[1], ZHANG Xin-le[1], YANG Gai-qing[3], XU Guo-wei[3*]
1. Department of Pharmacy, The 7th People's Hospital of Zhengzhou, Zhengzhou 450016, Henan, China
2. Department of Pharmaceutical Analysis, School of Pharmacy, Zhengzhou University, Zhengzhou 450000, Henan, China
3. Department of Neurology, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou 450001, Henan, China

［**Abstract**］ **Objective**　To investigate the effects of tirofiban on neuron injury in acute cerebral infarction (ACI) rats through silent information regulator factor 2-related enzyme 1 (SIRT1)/vascular endothelial growth factor (VEGF) signaling pathway. **Methods**　Seventy-five SD rats were randomly divided into sham group, model group, tirofiban (60 μg/kg) group, SIRT1 inhibitor (5 mg/kg SIRT1 specific inhibitor EX-527) group, and tirofiban＋SIRT1 inhibitor group, with 15 rats in each group. Except for the sham group, ACI models were constructed in the other 4 groups. The rats in each group were scored for neurological function. Triphenyl tetrazolium chloride staining was used to analyze the cerebral infarction volume percentage of rats. The serum malondialdehyde level was measured by thiobarbituric acid method, glutathione peroxidase (GSH-Px) level was detected by colorimetry, and superoxide dismutase (SOD) level was detected by microplate test. Hematoxylin-eosin staining was used to detect the pathological changes of rat brain tissue. Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay was used to detect the apoptosis of rat neurons. Western blotting was used to detect the expression of SIRT1 and VEGF proteins in the hippocampal tissue of rats. **Results**　Compared with the sham group, the pathological damage of the hippocampus was more serious in the model group, the neurological function score, cerebral infarction volume percentage, serum malondialdehyde level and neuronal cell apoptosis rate were significantly increased

［收稿日期］ 2022-07-01　　［接受日期］ 2022-11-14
［基金项目］ 河南省医学科技攻关计划联合共建项目（LHGJ20200763）. Supported by Joint Construction Project of Henan Medical Science and Technology Research Plan (LHGJ20200763).
［作者简介］ 王文文，硕士，副主任药师. E-mail: wangwenwen0285@163.com
*通信作者（Corresponding author）. Tel: 0371-89906876, E-mail: 25502689@qq.com

(all $P<0.05$), and the levels of serum GSH-Px and SOD and the expression levels of SIRT1 and VEGF proteins in the hippocampal tissue were significantly decreased (all $P<0.05$). Compared with the model group, the pathological damage of the hippocampus was decreased in the tirofiban group and tirofiban＋SIRT1 inhibitor group, the neurological function scores, cerebral infarction volume percentage, serum malondialdehyde levels and neuronal cell apoptosis rates were significantly decreased (all $P<0.05$), and the levels of serum GSH-Px and SOD and the expression levels of SIRT1 and VEGF proteins in the hippocampal tissue were significantly increased (all $P<0.05$); however, the corresponding indexes of the rats in the SIRT1 inhibitor group showed an opposite trend (all $P<0.05$). **Conclusion** Tirofiban may inhibit oxidative stress and neuron apoptosis by activating SIRT1/VEGF signaling pathway, thus alleviating neuron injury in ACI rats.

［**Key words**］ tirofiban; acute cerebral infarction; silent information regulator factor 2-related enzyme 1; vascular endothelial growth factor; neuron injury; apoptosis; oxidative stress

［Acad J Naval Med Univ, 2023, 44(6): 672-678］

急性脑梗死（acute cerebral infarction，ACI）是临床常见的心、脑血管和神经系统疾病，发病率和病死率高，逐渐成为公共卫生面临的主要挑战之一[1]。ACI 是脑血流短暂或永久减少的结果，仅涉及脑大动脉的范围[2]。目前，重组组织型纤溶酶原激活剂（recombinant tissue plasminogen activator，rt-PA）被认为是临床治疗 ACI 最有效的药物之一，但不良反应较多[3]。我国指南建议 ACI 患者发病 3 h 内应尽快静脉给予 rt-PA 溶栓治疗，发病 6 h 的 ACI 患者若不能使用 rt-PA，可考虑静脉给予尿激酶[4]。替罗非班（tirofiban）是一种高选择性的糖蛋白Ⅱb/Ⅲa 受体短效非肽抑制剂，可改善 ACI 患者的神经功能和体内炎症反应[5]，但其作用机制尚未明确。沉默信息调节因子 2 相关酶 1（silent information regulator factor 2-related enzyme 1，SIRT1）是一种依赖于烟酰胺腺嘌呤二核苷酸的Ⅲ类组蛋白脱乙酰酶，在所有细胞类型中都有表达。它以广泛的转录因子为靶标，参与许多生理和病理过程如氧化应激、炎症和凋亡等[6-8]。研究表明，SIRT1 通过调节血管内皮生长因子（vascular endothelial growth factor，VEGF）的表达参与脑缺血再灌注损伤过程[9]。本研究通过构建 ACI 大鼠模型，观察替罗非班能否通过影响 SIRT1/VEGF 信号通路对 ACI 大鼠起保护作用。

1 材料和方法

1.1 *实验动物* 75 只体重 220～270 g 的雄性 SD 大鼠购自珠海百试通生物科技有限公司［动物生产许可证号为 SCXK（粤）2020-0051］。将 SD 大鼠（4 只 / 笼）在（24±1）℃、光 / 暗循环（12 h/12 h）条件下饲养，予自由饮食、饮水。本研究通过郑州市第七人民医院动物伦理委员会审批，实验操作均遵循动物实验 3R 原则。

1.2 *试剂与仪器* 替罗非班（纯度 98%）购自上海源叶生物科技有限公司，SIRT1 特异性抑制剂 EX-527 和 ECL 发光试剂盒购自上海碧云天生物技术有限公司，丙二醛（硫代巴比妥酸法）、谷胱甘肽过氧化物酶（glutathione peroxidase，GSH-Px）（比色法）、超氧化物歧化酶（superoxide dismutase，SOD）（微板法）检测试剂盒及氯化三苯基四氮唑（triphenyl tetrazolium chloride，TTC）染色、H-E 染色试剂盒购自北京索莱宝科技有限公司，兔源 SIRT1、VEGF、GAPDH 一抗及 HRP 偶联的二抗、TUNEL 检测试剂盒购自英国 Abcam 公司。BX61 型光学显微镜购自日本 Olympus 公司，Gel Doc XR＋凝胶成像系统购自美国 BIO-RAD 公司。

1.3 *实验方法*

1.3.1 *分组及给药* 大鼠随机分为假手术组、模型组、替罗非班组、SIRT1 抑制剂组、替罗非班＋SIRT1 抑制剂组，每组 15 只。除假手术组外，其他 4 组大鼠均采用大脑中动脉闭塞法（middle cerebral artery occlusion，MCAO）诱发 ACI。替罗非班组大鼠术前 3 d 经尾静脉注射 60 μg/kg 替罗非班[10]；SIRT1 抑制剂组大鼠术前 3 d 予 5 mg/kg EX-527 腹腔注射[11]；替罗非班＋SIRT1 抑制剂组大鼠术前 3 d 经尾静脉注射 60 μg/kg 替罗非班的同时予 5 mg/kg EX-527 腹腔注射；假手术组和模型组大鼠术前 3 d 分别通过尾静脉和腹腔注射等体积的生理盐水。

1.3.2 *ACI 模型的建立*[12] 使用戊巴比妥钠（40 mg/kg）麻醉大鼠后，仰卧位固定，使体核温度保持在 37 ℃。将大鼠右侧颈部切开，充分露出右侧颈总动脉，分离并结扎颈外动脉及其分支。经颈动脉残端将 3-0 尼龙线插入颈内动脉，并延伸至

大脑前动脉以阻断大脑中动脉。2 h 后取出尼龙线使血流恢复，然后缝合皮肤、消毒，单笼饲养。假手术组除未阻断大脑中动脉外，其余手术操作与其他 4 组相同。各组大鼠于再灌注 24 h 后处死并进行取材和指标检测。

1.3.3　神经功能评分　使用双盲法进行神经功能评分[13]。评分标准：0 分，无神经损伤症状；1 分，患侧前爪不能完全伸出；2 分，行走时患侧前爪向内旋转；3 分，行走时患侧前爪向内倾斜；4 分，患侧足不能自主活动，失去意识；5 分，患侧肢体完全不能动。得分＞3 分说明 ACI 模型建立成功。

1.3.4　TTC 染色检测脑梗死体积百分数　神经功能评分完成后，每组取 5 只大鼠脑组织，切成 2 mm 厚的冠状切片，置于 2% TTC 溶液中在 37 ℃孵育 20 min，去除过量的 TTC 溶液，用 4% 多聚甲醛溶液固定。拍照并使用 ImageJ 1.8.0 软件分析脑梗死体积百分数。

1.3.5　血清丙二醛、GSH-Px、SOD 的测定　收集各组大鼠的外周血 2 mL，分别按照试剂盒说明书操作，检测丙二醛、GSH-Px、SOD 水平。

1.3.6　H-E 染色检测脑组织海马区病理变化　每组取 5 只大鼠脑组织，用 4% 多聚甲醛溶液固定过夜，经石蜡包埋后，用切片机切成 4 μm 厚的切片，经 H-E 染色后进行二甲苯透明、树胶封片。在光学显微镜下观察大鼠脑组织海马区病理变化并拍照。

1.3.7　TUNEL 法检测神经元凋亡情况　取脑组织海马区切片，用二甲苯脱蜡，乙醇梯度脱水至水化，按照 TUNEL 检测试剂盒说明书进行染色操作，然后在光学显微镜下随机选择 5 个视野拍照并进行细胞计数。正常细胞核呈蓝色，棕黄色为阳性细胞，按公式计算细胞凋亡率：细胞凋亡率（%）＝阳性细胞数 / 总细胞数 ×100%。

1.3.8　蛋白质印迹法检测 SIRT1/VEGF 通路相关蛋白表达　每组取 5 只大鼠脑组织海马区，置于－80 ℃保存。取出后研磨成匀浆，利用预冷的 RIPA 裂解缓冲液提取总蛋白质，采用 BCA 法测定蛋白质浓度，10% SDS-PAGE 分离蛋白质后转膜，然后用 5% 脱脂奶粉溶液孵育 2 h 以阻断非特异性结合位点。加入 SIRT1 一抗（稀释比例为 1 ∶ 1 000）、VEGF 一抗（稀释比例为 1 ∶ 1 000）、GAPDH 一抗（稀释比例为 1 ∶ 3 000），在 4 ℃ 孵育过夜。用 TBST 洗涤 2 次后，加入 HRP 偶联的二抗在室温下孵育 2 h，通过 ECL 发光试剂盒检测蛋白质显色情况。以 GAPDH 为内参照，采用凝胶成像系统分析目的蛋白的相对表达量。

1.4　统计学处理　应用 SPSS 25.0 软件进行统计学分析。计量资料以 $\bar{x}\pm s$ 表示，多组间比较采用单因素方差分析，多重比较采用 SNK-q 检验。检验水准（α）为 0.05。

2　结　果

2.1　替罗非班降低大鼠脑梗死体积百分数及神经功能评分　TTC 染色结果显示，假手术组大鼠脑组织呈均一红色，模型组、替罗非班组、SIRT1 抑制剂组、替罗非班＋SIRT1 抑制剂组大鼠脑组织存在白色梗死区域（图 1A）。由图 1B 可见，与假手术组比较，模型组大鼠脑梗死体积百分数升高（P＜0.05）；与模型组比较，替罗非班组、替罗非班＋SIRT1 抑制剂组大鼠脑梗死体积百分数降低，SIRT1 抑制剂组大鼠脑梗死体积百分数升高（P 均＜0.05）；与替罗非班组比较，替罗非班＋SIRT1 抑制剂组大鼠脑梗死体积百分数升高（P＜0.05）。由图 1C 可见，与假手术组相比，模型组大鼠的神经功能评分升高（P＜0.05）；与模型组比较，替罗非班组、替罗非班＋SIRT1 抑制剂组大鼠的神经功能评分降低，SIRT1 抑制剂组大鼠的神经功能评分升高（P 均＜0.05）；与替罗非班组比较，替罗非班＋SIRT1 抑制剂组大鼠的神经功能评分升高（P＜0.05）。

2.2　替罗非班抑制大鼠氧化应激　与假手术组相比，模型组大鼠血清丙二醛水平升高，GSH-Px、SOD 水平降低（P 均＜0.05）；与模型组相比，替罗非班组、替罗非班＋SIRT1 抑制剂组大鼠血清丙二醛水平降低，GSH-Px、SOD 水平升高，而 SIRT1 抑制剂组大鼠血清丙二醛水平升高，GSH-Px、SOD 水平降低（P 均＜0.05）；与替罗非班组比较，替罗非班＋SIRT1 抑制剂组大鼠血清丙二醛水平升高，GSH-Px、SOD 水平降低（P 均＜0.05）。见图 2。

2.3　替罗非班减轻大鼠脑组织海马区病理损伤程度　H-E 染色结果显示，假手术组大鼠脑组织海马区细胞结构完整，分布均匀，未见明显异常；模型组大鼠海马区大部分细胞排列紊乱，细胞间隙增大、细胞核破裂、凝缩变成固缩核，细胞坏死严重；替罗非班组大鼠海马区有少量细胞肿胀及坏死，病理损伤与模型组比较有所缓解；SIRT1 抑制

剂组大鼠海马区病理损伤程度较模型组明显加重；替罗非班＋SIRT1 抑制剂组大鼠脑组织海马区细胞排列紊乱，细胞间隙增大，大量细胞坏死，病变程度较替罗非班组严重，但较模型组有所缓解。见图 3。

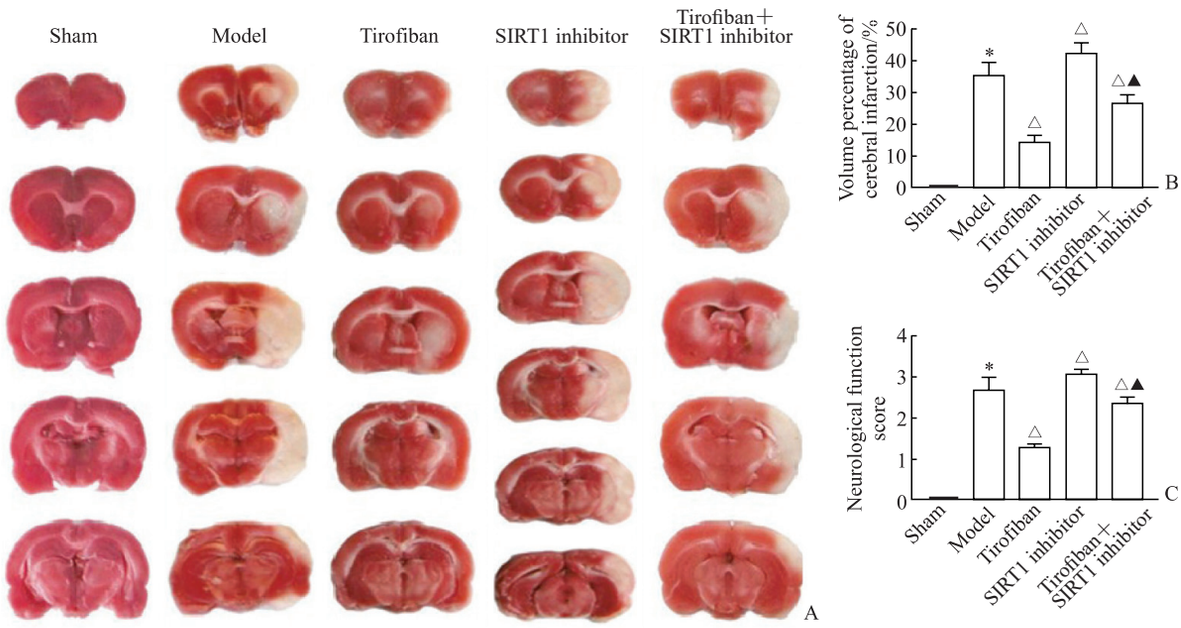

图 1　各组大鼠脑组织 TTC 染色、脑梗死体积百分数及神经功能评分

Fig 1　Brain tissue TTC staining, cerebral infarction volume percentage and neurological function score of rats in each group

A: Representative TTC staining pictures of brain tissue; B: Comparison of cerebral infarction volume percentages in each group; C: Comparison of neurological function scores in each group. Sham group: The rats undergoing simulated surgery were injected with normal saline via tail vein 3 d before surgery; Model group: ACI model rats were injected intraperitoneally with normal saline 3 d before modeling; Tirofiban group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein 3 d before modeling; SIRT1 inhibitor group: ACI model rats were injected intraperitoneally with SITR1-specific inhibitor EX-527 (5 mg/kg) 3 d before modeling; Tirofiban＋SITR1 inhibitor group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein and intraperitoneally administered with EX-527 (5 mg/kg) 3 d before modeling. $^{*}P<0.05$ vs sham group; $^{\triangle}P<0.05$ vs model group; $^{\blacktriangle}P<0.05$ vs tirofiban group. $n=5$ (B) or 15 (C), $\bar{x}\pm s$. TTC: Triphenyl tetrazolium chloride; ACI: Acute cerebral infarction; SITR1: Silent information regulator factor 2-related enzyme 1.

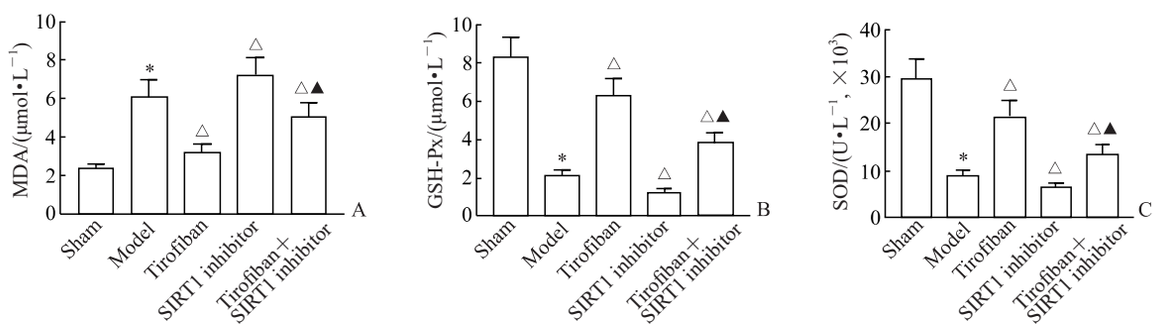

图 2　各组大鼠血清 MDA、GSH-Px、SOD 水平比较

Fig 2　Comparison of MDA, GSH-Px and SOD levels in serum of rats in each group

A: MDA level detected by thiobarbituric acid method; B: GSH-Px level detected by colorimetry; C: SOD level detected by microplate test. Sham group: The rats undergoing simulated surgery were injected normal saline via tail vein 3 d before surgery; Model group: ACI model rats were injected intraperitoneally normal saline 3 d before modeling; Tirofiban group: ACI model rats were injected tirofiban (60 μg/kg) via tail vein 3 d before modeling; SIRT1 inhibitor group: ACI model rats were injected intraperitoneally SIRT1-specific inhibitor EX-527 (5 mg/kg) 3 d before modeling; Tirofiban＋SIRT1 inhibitor group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein and intraperitoneally administered with EX-527 (5 mg/kg) 3 d before modeling. $^{*}P<0.05$ vs sham group; $^{\triangle}P<0.05$ vs model group; $^{\blacktriangle}P<0.05$ vs tirofiban group. $n=5$, $\bar{x}\pm s$. MDA: Malondialdehyde; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase; ACI: Acute cerebral infarction; SIRT1: Silent information regulator factor 2-related enzyme 1.

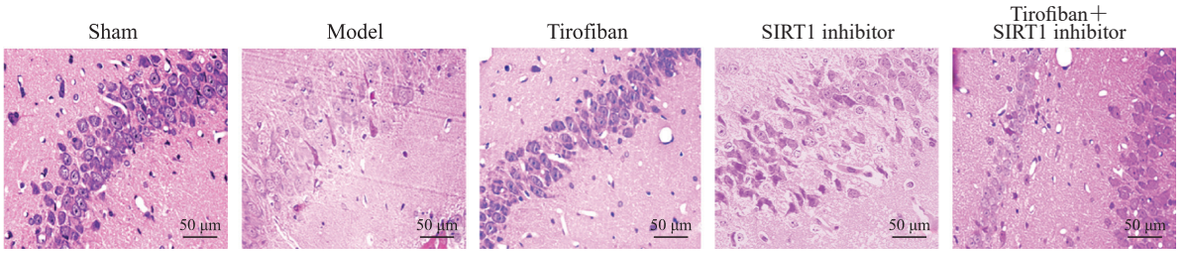

图 3　各组大鼠脑组织海马区苏木精－伊红染色

Fig 3　Hematoxylin-eosin staining of hippocampus of rats in each group

Sham group: The rats undergoing simulated surgery were injected with normal saline via tail vein 3 d before surgery; Model group: ACI model rats were injected intraperitoneally with normal saline 3 d before modeling; Tirofiban group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein 3 d before modeling; SIRT1 inhibitor group: ACI model rats were injected intraperitoneally with SIRT1-specific inhibitor EX-527 (5 mg/kg) 3 d before modeling; Tirofiban＋SIRT1 inhibitor group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein and intraperitoneally administered with EX-527 (5 mg/kg) 3 d before modeling. ACI: Acute cerebral infarction; SIRT1: Silent information regulator factor 2-related enzyme 1.

2.4　替罗非班抑制大鼠脑组织海马区的神经元凋亡　TUNEL 染色结果显示，与假手术组相比，模型组大鼠脑组织海马区神经元凋亡率升高（P＜0.05）；与模型组比较，替罗非班组、替罗非班＋SIRT1 抑制剂组大鼠脑组织海马区神经元凋亡率降低，SIRT1 抑制剂组大鼠脑组织海马区神经元凋亡率升高（P均＜0.05）；与替罗非班组比较，替罗非班＋SIRT1 抑制剂组大鼠脑组织海马区神经元凋亡率升高（P＜0.05）。见图 4。

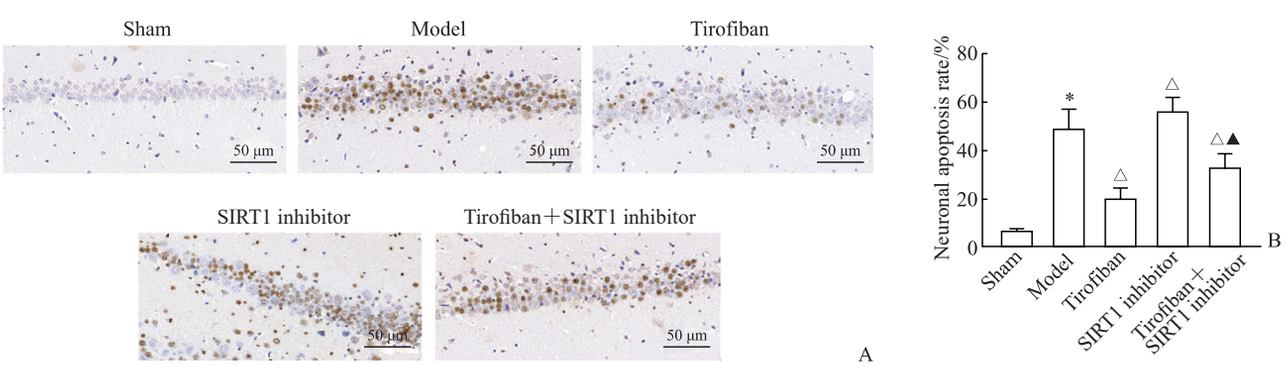

图 4　TUNEL 染色检测各组大鼠脑组织海马区神经元凋亡情况

Fig 4　Neuronal apoptosis in brain hippocampal regions in rats of each group detected by TUNEL staining

A: Representative TUNEL staining pictures of brain hippocampal regions of rats in each group; B: Comparison of neuronal apoptosis rate in each group. Sham group: The rats undergoing simulated surgery were injected with normal saline via tail vein 3 d before surgery; Model group: ACI model rats were injected intraperitoneally with normal saline 3 d before modeling; Tirofiban group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein 3 d before modeling; SIRT1 inhibitor group: ACI model rats were injected intraperitoneally with SIRT1-specific inhibitor EX-527 (5 mg/kg) 3 d before modeling; Tirofiban＋SIRT1 inhibitor group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein and intraperitoneally administered with EX-527 (5 mg/kg) 3 d before modeling. $^{*}P$＜0.05 vs sham group; $^{\triangle}P$＜0.05 vs model group; $^{\blacktriangle}P$＜0.05 vs tirofiban group. n＝5, $\bar{x}\pm s$. TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; ACI: Acute cerebral infarction; SIRT1: Silent information regulator factor 2-related enzyme 1.

2.5　替罗非班激活 SIRT1/VEGF 信号通路　蛋白质印迹法检测结果显示，与假手术组相比，模型组大鼠海马组织中 SIRT1、VEGF 蛋白表达水平均降低（P均＜0.05）；与模型组比较，替罗非班组、替罗非班＋SIRT1 抑制剂组大鼠海马组织中 SIRT1、VEGF 蛋白表达水平均升高，SIRT1 抑制剂组大鼠海马组织中 SIRT1、VEGF 蛋白表达水平均降低（P均＜0.05）；与替罗非班组比较，替罗非班＋SIRT1 抑制剂组大鼠海马组织中 SIRT1、VEGF 蛋白表达水平均降低（P均＜0.05）。见图 5。

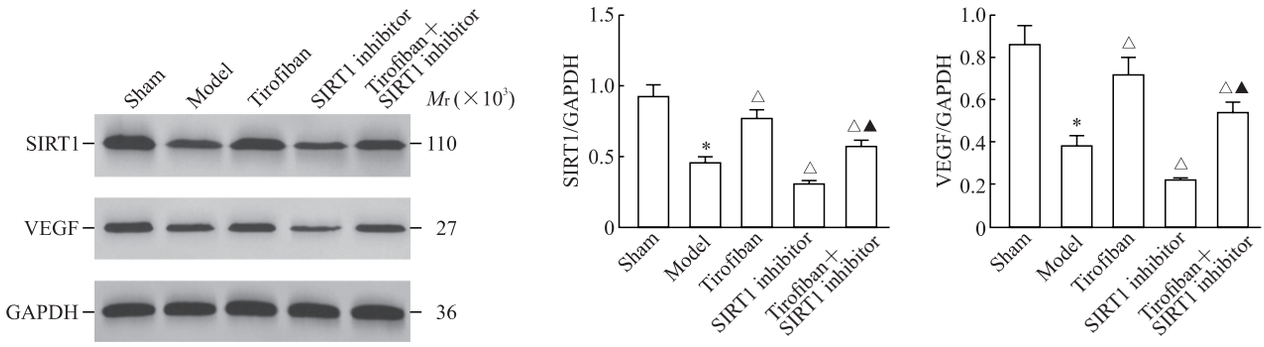

图 5　蛋白质印迹法检测各组大鼠海马组织中 SIRT1、VEGF 蛋白表达

Fig 5　Expression of SIRT1 and VEGF proteins in hippocampal tissue of rats in each group detected by Western blotting

Sham group: The rats undergoing simulated surgery were injected with normal saline via tail vein 3 d before surgery; Model group: ACI model rats were injected intraperitoneally with normal saline 3 d before modeling; Tirofiban group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein 3 d before modeling; SIRT1 inhibitor group: ACI model rats were injected intraperitoneally with SIRT1-specific inhibitor EX-527 (5 mg/kg) 3 d before modeling; Tirofiban＋inhibitor group: ACI model rats were injected with tirofiban (60 μg/kg) via tail vein and intraperitoneally administered with EX-527 (5 mg/kg) 3 d before modeling. $^{*}P<0.05$ vs sham group; $^{\triangle}P<0.05$ vs model group; $^{\blacktriangle}P<0.05$ vs tirofiban group. $n=5$, $\bar{x}\pm s$. SIRT1: Silent information regulator factor 2-related enzyme 1; VEGF: Vascular endothelial growth factor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; ACI: Acute cerebral infarction.

3　讨　论

大脑是高灌注、高耗氧量、高代谢和低能量储备的重要器官。发生 ACI 时，血管内血流中断导致缺氧缺血、梗死区缺氧、脑组织能量耗尽，最终导致脑组织坏死或软化。研究表明，MCAO 可导致大鼠的行为、神经化学和组织学异常，该模型能够模拟人类 ACI 的许多特征[14-15]。本研究通过 MCAO 构建 ACI 模型，考察替罗非班对 ACI 的神经保护作用，结果证实替罗非班可抑制氧化应激损伤、神经元凋亡，调节 SIRT1、VEGF 蛋白表达。

脑缺血是导致神经功能损害的重要原因。神经功能缺损评分是评价脑损伤的常用指标[16]。本研究结果表明，闭塞大鼠大脑中动脉可导致神经功能缺损评分升高，而替罗非班可改善神经功能、减轻脑缺血的发展；ACI 模型大鼠出现神经元受损，替罗非班可改善脑损伤、缩小梗死灶。以上结果提示替罗非班能抑制 MCAO 诱导的神经元损伤，对脑缺血有保护作用。

人体大约 20% 的氧气供给被脑组织利用。大量证据表明，脑氧化应激在 ACI 的发病中起着重要作用。SOD 是抵御线粒体基质中超氧阴离子的主要防线[17]。GSH-Px 是一种细胞内元件，在保护细胞免受氧化损伤方面起着至关重要的作用[18]。丙二醛是脂质过氧化的重要产物，可以间接反映细胞内的脂质过氧化水平[19]。缺血的大脑皮质抗氧化酶活性升高，脂质过氧化产物含量降低，表明脑抗氧化能力提高。本研究结果显示替罗非班提高了 SOD 和 GSH-Px 水平、降低了丙二醛含量，与既往研究[14]一致，表明替罗非班能够通过抑制氧化应激减轻脑损伤。

SIRT1 参与氧化应激、自噬、神经保护和线粒体功能等多种生物学过程[20]。SIRT1 在脑缺血再灌注大鼠脑组织中低表达，激活其表达能改善神经功能障碍[21]。VEGF 由大脑中许多神经血管细胞产生和分泌，被认为是缺血后血管生成的关键因子[22]。缺血性脑卒中发生时 SIRT1 表达降低，下游 VEGF 表达减少，阻碍脑血管生成，从而使神经功能受损[9]。EX-527 是 SIRT1 的特异性抑制剂[23]。本研究中 EX-527 抑制 ACI 大鼠 SIRT1 表达的同时降低了 VEGF 蛋白水平，增加了脑组织病理损伤、神经元凋亡，激活了大鼠体内的氧化应激反应；抑制 SIRT1 减弱了替罗非班对缺血再灌注损伤的保护作用，提示替罗非班通过激活 SIRT1/VEGF 信号通路对 ACI 大鼠的神经元损伤起到保护作用。

综上所述，替罗非班可能通过激活 SIRT1/VEGF 信号通路抑制 ACI 过程中的氧化应激及神经元损伤，发挥对脑的保护作用。

［参 考 文 献］

［1］ ZHEN X Y, ZHENG Y, HONG X N, CHEN Y, GU P, TANG J R, et al. Physiological ischemic training promotes

brain collateral formation and improves functions in patients with acute cerebral infarction[J/OL]. Front Neurol, 2016, 7: 235. DOI: 10.3389/fneur.2016.00235.

[2] ZHANG J C, WU Y, WENG Z L, ZHOU T, FENG T, LIN Y. Glycyrrhizin protects brain against ischemia-reperfusion injury in mice through HMGB1-TLR4-IL-17A signaling pathway[J]. Brain Res, 2014, 1582: 176-186.

[3] DONG M X, HU Q C, SHEN P, PAN J X, WEI Y D, LIU Y Y, et al. Recombinant tissue plasminogen activator induces neurological side effects independent on thrombolysis in mechanical animal models of focal cerebral infarction: a systematic review and meta-analysis[J/OL]. PLoS One, 2016, 11: e0158848. DOI: 10.1371/journal.pone.0158848.

[4] 中华医学会神经病学分会，中华医学会神经病学分会脑血管病学组. 中国急性缺血性脑卒中诊治指南2018[J]. 中华神经科杂志，2018，51：666-682.

[5] 田雨，胡晓. 替罗非班在急性脑梗死中应用的研究进展[J]. 中国医药，2021，16：763-767.

[6] LEE I C, HO X Y, GEORGE S E, GOH C W, SUNDARAM J R, PANG K K L, et al. Oxidative stress promotes SIRT1 recruitment to the GADD34/PP1α complex to activate its deacetylase function[J]. Cell Death Differ, 2018, 25: 255-267.

[7] VELAGAPUDI R, EL-BAKOUSH A, LEPIARZ I, OGUNRINADE F, OLAJIDE O A. AMPK and SIRT1 activation contribute to inhibition of neuroinflammation by thymoquinone in BV2 microglia[J]. Mol Cell Biochem, 2017, 435: 149-162.

[8] ZHANG X S, LU Y, LI W, TAO T, PENG L, WANG W H, et al. Astaxanthin ameliorates oxidative stress and neuronal apoptosis via SIRT1/NRF2/Prx2/ASK1/p38 after traumatic brain injury in mice[J]. Br J Pharmacol, 2021, 178: 1114-1132.

[9] ZHENG X W, SHAN C S, XU Q Q, WANG Y, SHI Y H, WANG Y, et al. *Buyang Huanwu* decoction targets SIRT1/VEGF pathway to promote angiogenesis after cerebral ischemia/reperfusion injury[J]. Front Neurosci, 2018, 12: 911.

[10] 刘玲梅，张梅，刘艳红，周欣，叶帆，何瑞波，等. 替罗非班对大鼠心肌缺血再灌注后无复流及NF-κB激活的影响[J]. 中国病理生理杂志，2009，25：1892-1897.

[11] 曹自为，张嵘，李蒙，管叶明，汪青松. 丹参多酚酸通过SIRT1/HMGB1路径改善大鼠脑缺血/再灌注损伤的机制研究[J]. 中风与神经疾病杂志，2021，38：4-8.

[12] YANG B B, ZOU M, ZHAO L, ZHANG Y K. Astaxanthin attenuates acute cerebral infarction via Nrf-2/HO-1 pathway in rats[J/OL]. Curr Res Transl Med, 2021, 69: 103271. DOI: 10.1016/j.retram.2020.103271.

[13] BEDERSON J B, PITTS L H, TSUJI M, NISHIMURA M C, DAVIS R L, BARTKOWSKI H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination[J]. Stroke, 1986, 17: 472-476.

[14] DUAN X H, WANG W L, LIU X Q, YAN H W, DAI R, LIN Q. Neuroprotective effect of ethyl acetate extract from *Gastrodia elata* against transient focal cerebral ischemia in rats induced by middle cerebral artery occlusion[J]. J Tradit Chin Med, 2015, 35: 671-678.

[15] PARK D J, KANG J B, KOH P O. Epigallocatechin gallate alleviates neuronal cell damage against focal cerebral ischemia in rats[J]. J Vet Med Sci, 2020, 82: 639-645.

[16] HUANG Y, PAN L S, WU T. Improvement of cerebral ischemia-reperfusion injury by *L*-3-*n*-butylphthalide through promoting angiogenesis[J]. Exp Brain Res, 2021, 239: 341-350.

[17] SUN K, FAN J Y, HAN J Y. Ameliorating effects of traditional Chinese medicine preparation, Chinese materia medica and active compounds on ischemia/reperfusion-induced cerebral microcirculatory disturbances and neuron damage[J]. Acta Pharm Sin B, 2015, 5: 8-24.

[18] YAN W J, REN D Q, FENG X X, HUANG J W, WANG D B, LI T, et al. Neuroprotective and anti-inflammatory effect of pterostilbene against cerebral ischemia/reperfusion injury via suppression of COX-2[J/OL]. Front Pharmacol, 2021, 12: 770329. DOI: 10.3389/fphar.2021.770329.

[19] YU J, WANG W N, MATEI N, LI X, PANG J W, MO J, et al. Ezetimibe attenuates oxidative stress and neuroinflammation via the AMPK/Nrf2/TXNIP pathway after MCAO in rats[J/OL]. Oxid Med Cell Longev, 2020, 2020: 4717258. DOI: 10.1155/2020/4717258.

[20] HORIO Y, HAYASHI T, KUNO A, KUNIMOTO R. Cellular and molecular effects of sirtuins in health and disease[J]. Clin Sci (Lond), 2011, 121: 191-203.

[21] SHI Y H, ZHANG X L, YING P J, WU Z Q, LIN L L, CHEN W, et al. Neuroprotective effect of astragaloside Ⅳ on cerebral ischemia/reperfusion injury rats through Sirt1/Mapt pathway[J/OL]. Front Pharmacol, 2021, 12: 639898. DOI: 10.3389/fphar.2021.639898.

[22] YIN K J, HAMBLIN M, CHEN Y E. Angiogenesis-regulating microRNAs and ischemic stroke[J]. Curr Vasc Pharmacol, 2015, 13: 352-365.

[23] QIN T T, LIU W H, HUO J F, LI L L, ZHANG X Y, SHI X L, et al. SIRT1 expression regulates the transformation of resistant esophageal cancer cells via the epithelial-mesenchymal transition[J]. Biomed Pharmacother, 2018, 103: 308-316.

［本文编辑］　尹　茶