[文章编号] 1671-587X(2024)05-1196-09 DOI:10.13481/j.1671-587X.20240502

人参皂苷Rg1对小鼠慢性间歇性缺氧诱导脑损伤的减轻作用及其机制

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[摘 要] **目的：**探讨人参皂苷Rg1对小鼠慢性间歇性缺氧（CIH）诱导脑损伤的减轻作用，并阐明其可能的作用机制。**方法：**40只C57BL/6雄性小鼠随机分为对照组、模型组、抑制剂组（给予钙蛋白酶1抑制剂）、低剂量人参皂苷Rg1组（给予10 mg·kg^{-1}人参皂苷Rg1）和高剂量人参皂苷Rg1组（给予20 mg·kg^{-1}人参皂苷Rg1）。除对照组外，其余各组小鼠均置于自动调节氧浓度的低氧舱中以诱导小鼠缺氧性脑损伤。检测各组小鼠尾外周血氧饱和度（SpO_2），采用Morris水迷宫实验检测各组小鼠逃逸潜伏期、路径长度和游泳路线穿过目标象限频次，采用试剂盒检测血清中血尿素氮（BUN）、乳酸（LA）、丙二醛（MDA）、白细胞介素6（IL-6）和肿瘤坏死因子α（TNF-α）水平以及血清中超氧化物歧化酶（SOD）和乳酸脱氢酶（LDH）活性，采用HE染色观察各组小鼠脑组织损伤程度，采用二氢乙啶（DHE）探针检测各组小鼠海马组织CA1区中活性氧（ROS）表达水平，采用Western blotting法检测各组小鼠脑组织中钙蛋白酶1、IL-6、和TNF-α蛋白表达水平。**结果：**与对照组比较，模型组小鼠SpO_2明显降低（$P<0.01$），表明模型建立成功。与模型组比较，抑制剂组和低及高剂量人参皂苷Rg1组小鼠SpO_2明显升高（$P<0.01$）。Morris水迷宫实验，与对照组比较，模型组小鼠逃逸潜伏期和路径长度明显延长（$P<0.01$），游泳路线穿过目标象限频次明显减少；与模型组比较，抑制剂组、低和高剂量人参皂苷Rg1组小鼠逃逸潜伏期和路径长度明显缩短，游泳路线穿过目标象限频次明显增多。与对照组比较，模型组小鼠血清中BUN、LA、MDA、IL-6和TNF-α水平明显升高（$P<0.01$），LDH活性明显升高（$P<0.01$），SOD活性明显降低（$P<0.01$）；与模型组比较，抑制剂组、低和高剂量人参皂苷Rg1组小鼠血清中BUN、LA、MDA、IL-6和TNF-α水平明显降低（$P<0.01$），LDH活性明显降低（$P<0.01$），SOD活性明显升高（$P<0.01$）。HE染色，与对照组比较，模型组小鼠海马组织CA1区中锥体神经元排列疏松，部分神经元呈三角形，核固缩，胞质浓染，少数神经元脱失，呈明显缺氧性神经元损伤形态；与模型组比较，抑制剂组、低和高剂量人参皂苷Rg1组小鼠海马组织CA1区中缺氧性神经元损伤表现有效改善。DHE探针检测，与对照组比较，模型组小鼠海马组织CA1区中ROS水平明显升高（$P<0.01$）；与模型组比较，抑制剂组、低和高剂量人参皂苷Rg1组小鼠海马组织CA1区中ROS水平明显降低（$P<0.01$）。Western blotting法检测，与对照组比较，模型组小鼠海马组织中钙蛋白酶1、TNF-α和IL-6蛋白表达水平明显升高（$P<0.01$）；与模型组比较，抑制剂组、低和高剂量人参皂苷Rg1组小鼠海马组织中钙蛋白酶1、TNF-α和IL-6蛋白表达水平明显降低（$P<0.01$）。**结论：**人参皂苷Rg1可减轻小鼠CIH诱导的脑组织损伤，其机制可能与抑制脑组织炎症反应和氧化应激并下调钙蛋白酶1表达有关。

[关键词] 人参皂苷Rg1；钙蛋白酶1；钙蛋白酶1抑制剂；慢性间歇性缺氧；脑损伤

[中图分类号] R965 [文献标志码] A

[收稿日期] 2023-10-30

[基金项目] 国家自然科学基金项目（81973553）

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Alleviative effect of ginsenoside Rg1 on brain injury induced by chronic intermittent hypoxia in mice and its mechanism

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ABSTRACT **Objective**: To discuss the alleviative effect of ginsenoside Rg1 on chronic intermittent hypoxia (CIH)-induced brain injury in the mice, and to clarify its possible mechanism. **Methods**: Forty male C57BL/6 mice were randomly divided into control group, model group, inhibitor group (treated with calpain-1 inhibitor), low dose of ginsenoside Rg1 group (treated with 10 $mg \cdot kg^{-1}$ ginsenoside Rg1), and high dose of ginsenoside Rg1 group (treated with 20 $mg \cdot kg^{-1}$ ginsenoside Rg1). Except for the control group, the mice in all other groups were placed in a hypoxic chamber with automatically regulated oxygen concentration to induce hypoxic brain injury. The peripheral blood oxygen saturation (SpO_2) of tail of the mice in various groups was detected; the escape latencies and path lengths and the frequency of swimming route crossing the target quadrant of the mice in various groups were determined by Morris water maze test; the levels of blood urea nitrogen (BUN), lactate (LA), malondialdehyde (MDA), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) and the activities of superoxide dismutase (SOD) and lactate dehydrogenase (LDH) in serum of the mice in various groups were detected by kits; the degrees of brain tissue injury of the mice in various groups were observed by HE staining. The levels of reactive oxygen species (ROS) in CA1 region of hippocampus tissue of the mice in various groups were detected by dihydroethidium (DHE) probe; the expression levels of calpain-1, IL-6, and TNF-α proteins in brain tissue of the mice in various groups were detected by Western blotting method. **Results**: Compared with control group, the SpO_2 of the mice in model group was significantly decreased ($P<0.01$), indicating that the model was successfully established. Compared with model group, the SpO_2 of the mice in inhibitor group, low dose of ginsenoside Rg1 group and high dose of ginsenoside Rg1 group were significantly increased ($P<0.01$). The Morris water maze test results showed that compared with control group, the escape latency and path length of the mice in model group were significantly prolonged ($P<0.01$), and the frequency of swimming route of crossing the target quadrant was significantly decreased; compared with model group, the escape latencies and path lengths of the mice in inhibitor group, low dose of ginsenoside Rg1 group and high dose of ginsenoside Rg1 group were significantly shortened ($P<0.01$), and the frequency of swimming route of crossing the target quadrant was significantly increased. Compared with control group, the levels of BUN, LA, MDA, IL-6, and TNF-α in serum of the mice in model group were significantly increased ($P<0.01$), while the activity of LDH was significantly increased ($P<0.01$), and the activity of SOD was significantly decreased ($P<0.01$); compared with model group, the levels of BUN, LA, MDA, IL-6, and TNF-α in serum of the mice in inhibitor group, low dose of ginsenoside Rg1 group and high dose of ginsenoside Rg1 group were significantly decreased ($P<0.01$), while the activities of LDH were significantly decreased ($P<0.01$), and the activities of SOD were significantly increased ($P<0.01$). The HE staining results showed that compared with control group, the pyramidal neurons in CA1 region of hippocampus tissue of the mice in model group were loosely arranged, while some neurons were triangular, with nuclear pyknosis, cytoplasmic hyperchromasia, and a few neurons were lost, indicating obvious hypoxic neuronal injury; compared with model group, the hypoxic neuronal injury in CA1 region in hippocampus tissue of the mice in inhibitor group, low dose of ginsenoside Rg1 group and

high dose of ginsenoside Rg1 group was effectively alleviated. The DHE probe detection showed that compared with control group, the level of ROS in CA1 region in hippocampus tissue of the mice in model group was significantly increased ($P<0.01$); compared with model group, the levels of ROS in CA1 region in hippocampus tissue of the mice in inhibitor group, low dose of ginsenoside Rg1 group and high dose of ginsenoside Rg1 group were significantly decreased ($P<0.01$). The Western blotting results showed that compared with control group, the expression levels of calpain-1, TNF-α, and IL-6 proteins in hippocampus tissue of the mice in model group were significantly increased ($P<0.01$); compared with model group, the expression levels of calpain-1, TNF-α, and IL-6 proteins in hippocampus tissue of the mice in inhibitor group, low dose of GRg1 group and high dose of GRg1 group were significantly decreased ($P<0.01$). **Conclusion**: Ginsenoside Rg1 can alleviate brain tissue injury of the mice induced by CIH; its mechanism may be related to the inhibition of brain tissue inflammatory response and oxidative stress, and the downregulation of calpain-1 expression.

KEYWORDS Ginsenoside Rg1; Calpain-1; Calpain-1 inhibitor; Chronic intermittent hypoxia; Brain injury

慢性间歇性缺氧（chronic intermittent hypoxia，CIH）是阻塞性睡眠呼吸暂停（obstructive sleep apnea，OSA）的主要特征，与多器官损伤有关[1]。近年来，OSA引起的大脑认知功能障碍受到广泛关注[2]。海马神经元变化在神经认知缺陷中发挥重要作用[3]。钙蛋白酶1是一种钙活化中性半胱氨酸蛋白酶[4]，被认为是诱导和维持神经炎症和氧化应激的最重要因素[5]。研究[6]显示：黄芪甲苷通过抑制钙蛋白酶1在缺氧诱导的海马神经元氧化应激和细胞凋亡方面起重要作用。人参皂苷Rg1是人参的主要药物活性成分提取物[7]。人参皂苷Rg1除可增强机体的抗氧化活性外，还具有抗炎和抑制细胞凋亡的作用[8]。研究[9]显示：人参皂苷Rg1在H_2O_2诱导的脑损伤炎症修复中发挥神经保护作用，但人参皂苷Rg1调节钙蛋白酶1是否在CIH诱导的脑损伤中发挥作用尚不清楚。本研究探讨人参皂苷Rg1调节钙蛋白酶1对CIH诱导的脑损伤炎症反应和氧化应激的作用机制。

1 材料与方法

1.1 实验动物、主要试剂和仪器 C57BL/6雄性健康小鼠40只，体质量20～30 g，购自辽宁长生生物技术股份有限公司，动物使用许可证号：SYXK（辽）2020-0001。人参皂苷Rg1，纯度≥98.0%，购自南京景竹生物科技有限公司，钙蛋白酶1抑制剂MDL-28170购自美国Sigma公司，HE染色试剂盒购自南京建成生物技术有限公司，二氢乙啶（dihydroethidium，DHE）检测试剂盒、放射免疫沉淀法裂解液和苯甲基磺酰氟购自上海碧云天生物技术有限公司，丙二醛（malondialdehyde，MDA）、超氧化物歧化酶（superoxide dismutase，SOD）、乳酸脱氢酶（lactate dehydrogenase，LDH）、乳酸（lactic acid，LA）、血尿素氮（blood urea nitrogen，BUN）测定试剂盒购自南京建成生物工程有限公司，白细胞介素6（interleukin-6，IL-6）和肿瘤坏死因子α（tumor necrosis factor，TNF-α）试剂盒购自上海酶联生物科技有限公司，钙蛋白酶1、IL-6和TNF-α一抗购自武汉三鹰生物技术有限公司，β-肌动蛋白一抗购自武汉爱博泰克生物科技有限公司，二喹啉甲酸试剂盒购自荷瑞生物科技有限公司，十二烷基硫酸钠、牛血清白蛋白和三羟基氨基甲烷购自北京鼎国昌盛生物技术有限责任公司，氯化钠购自天津博迪化工股份有限公司，三羟甲基氨基甲烷吐温20缓冲液（tris-buffered saline with Tween 20，TBST）购自天津市大茂化学试剂厂。干燥箱购自上海福玛实验有限公司，荧光显微镜购自德国徕卡公司，Bio-Rad电泳系统和半干转膜仪购自美国Bio-Rad公司，自动凝胶成像系统购自英国Syngene公司，DNM-9602G酶标分析仪购自北京普朗新技术有限公司，动物间歇氧浓度控制系统购自上海塔望智能科技有限公司。

1.2 实验分组和CIH模型制备 40只C57BL/6雄性健康小鼠随机分为对照组、模型组（制备CIH模型）、抑制剂组（给予钙蛋白酶1抑制剂）、低剂量人参皂苷Rg1组（给予10 mg·kg^{-1}人参皂苷Rg1）和高剂量人参皂苷Rg1（给予20 mg·kg^{-1}人参皂苷Rg1）。除对照组外，其余各组小鼠上午8时至下午4时置入自动调节氧浓度的低氧舱中，每天8 h，每

个间歇性缺氧周期为90 s低氧期＋90 s复氧期，通过开放或压缩氮气管道使低氧舱内氧浓度由21%下降至约10%，再维持至21%。28 d后检测小鼠尾周围血氧饱和度（oxygen saturation，SpO_2），SpO_2在60%～85%视为CIH模型构建成功。建模期间，低和高剂量人参皂苷Rg1组小鼠每天分别给予10和20 mg·kg^{-1}人参皂苷Rg1 0.2 mL灌胃；抑制剂组小鼠每天腹腔注射20 mg·kg^{-1}钙蛋白酶1抑制剂MDL-28170 0.2 mL，对照组小鼠在相同条件下置入含常氧气体的饲养笼。

1.3　各组小鼠SpO_2检测　经过28 d造模后，采用血氧饱和度仪夹住各组鼠尾，检测各组小鼠SpO_2，根据血氧饱和度仪记录的SpO_2值评估小鼠CIH情况。

1.4　Morris水迷宫实验检测各组小鼠学习记忆能力　Morris水迷宫实验为期6 d，在SpO_2检测后进行。各组小鼠上午探索2个象限，下午探索另外2个象限，标记各个象限中间点位置为小鼠入水点。第1～2天检测小鼠的运动能力和视觉条件。小鼠60 s内成功登上目标平台停留5 s，直到60 s测试结束（若小鼠58 s成功登上目标平台，在目标平台停留5 s才结束测试），若小鼠60 s内未成功登上目标平台，人为将小鼠置于平台停留5 s。第3～5天检测小鼠的逃逸潜伏期和路径长度。小鼠60 s内成功登上目标平台停留5 s，测试结束，若小鼠60 s内未成功登上目标平台，60 s测试结束。第6天检测小鼠游泳路线穿过目标象限频次。移除目标平台，小鼠入水游行60 s测试结束。导出数据，分析各组小鼠逃逸潜伏期、路径长度和穿过目标象限频次，判断小鼠学习记忆能力。

1.5　试剂盒检测各组小鼠血清中BUN、LA、MDA、IL-6和TNF-α水平及LDH和SOD活性　采用20%乌拉坦麻醉后，各组小鼠眼底静脉丛取血，全血4 ℃静置30 min，3 000 r·min^{-1}离心15 min，提取上清。按照相应试剂盒说明书步骤分别检测小鼠血清中BUN、LA、MDA、IL-6和TNF-α水平及LDH和SOD活性。

1.6　HE染色观察各组小鼠海马组织CA1区锥体神经元形态表现　处死小鼠，将各组小鼠完整脑组织固定于4%多聚甲醛中。24 h后根据小鼠海马位置结构对脑组织进行冠状切片，包埋于石蜡中，制备脑组织切片后进行HE染色，观察小鼠海马组织CA1区锥体神经元形态表现。

1.7　DHE荧光探针检测各组小鼠海马组织CA1区中活性氧（reactive oxygen species，ROS）水平　各组小鼠脑组织切片置于60 ℃干燥箱中烘干，经二甲苯和梯度乙醇洗脱，滴加DHE染色液（5 μmol·L^{-1}），37 ℃水浴孵育30 min，磷酸盐缓冲液（phosphate buffered saline，PBS）洗涤3次，每次5 min，在荧光显微镜下观察，采用Image J软件分析各组小鼠海马组织CA1区中ROS水平，ROS水平＝ROS荧光细胞数/细胞总数×100%。

1.8　Western blotting法检测小鼠海马区组织中钙蛋白酶1、IL-6和TNF-α蛋白表达水平　取各组小鼠海马区组织于0.1 g于1 mL裂解缓冲液（放射免疫沉淀法裂解液＋1%苯甲基磺酰氟）中裂解并均匀化，通过二喹啉甲酸试剂盒测定蛋白质浓度制备蛋白样品。采用十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（8%～12%聚丙烯酰胺凝胶）分离蛋白样品，置于转模仪中转移到聚偏氟乙烯膜上，采用1%牛血清白蛋白封闭膜1.5 h，然后与钙蛋白酶1、IL-6、TNF-α和β-肌动蛋白一抗4 ℃孵育过夜。次日，采用TBST洗涤膜3次，每次5 min，然后与相应二抗室温孵育1 h，TBST摇床清洗3次，每次5 min。采用Image J软件分析蛋白表达灰度值，计算目的蛋白表达水平。目的蛋白表达水平＝目的蛋白条带灰度值/内参β-肌动蛋白条带灰度值。

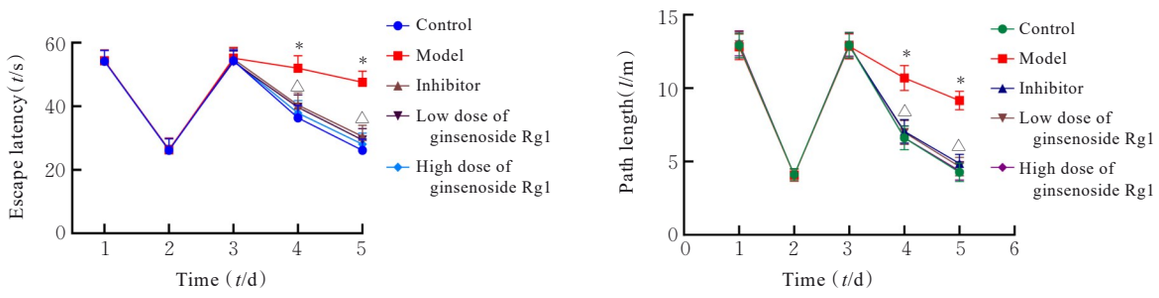
1.9　统计学分析　采用SPSS 26.0统计软件进行统计学分析。各组小鼠SpO_2，血清中BUN、LA、MDA、IL-6和TNF-α水平及LDH和SOD活性，海马组织CA1区中ROS水平，海马组织中钙蛋白酶1、IL-6和TNF-α蛋白表达水平均符合正态分布，以$\bar{x}\pm s$表示；各组小鼠逃逸潜伏期和路径长度多组间比较采用单因素方差分析，组间两两比较采用t检验。以$P<0.05$为差异有统计学意义。

2　结　果

2.1　各组小鼠SpO_2水平　与对照组（91.8%±4.4%）比较，模型组小鼠SpO_2（66.1%±2.9%）明显降低（$P<0.01$），表明模型建立成功。与模型组比较，抑制剂组（85.5%±3.1%）、低剂量人参皂苷Rg1组（86.1%± 3.9%）和高剂量人参皂苷Rg1组小鼠SpO_2（89.9%±4.2%）明显升高（$P<0.01$）。

2.2　各组小鼠学习记忆能力　与对照组比较，模型组小鼠逃逸潜伏期和路径长度明显延长（$P<0.01$），

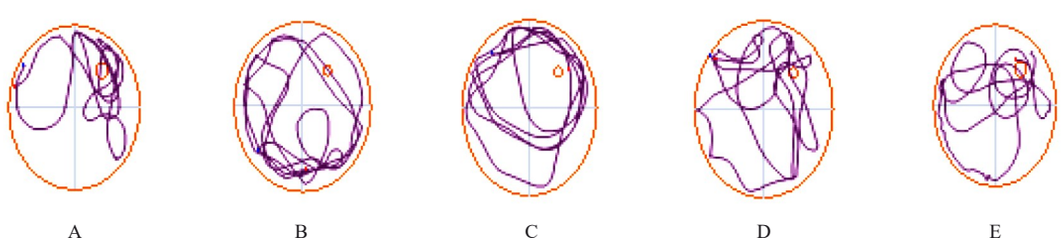
游泳路线穿过目标象限频次明显减少；与模型组比较，抑制剂组和低及高剂量人参皂苷 Rg1 组小鼠逃逸潜伏期及路径长度明显缩短（$P<0.01$），游泳路线穿过目标象限频次明显增多。见图 1 和 2。



*$P<0.01$ compared with control group; △$P<0.01$ compared with model group.

图 1　各组小鼠逃逸潜伏期(A)和路径长度(B)

Fig. 1　Escape latencies(A) and path lengths(B) of mice in various groups



A: Control group; B: Model group; C: Inhibitor group; D: Low dose of ginsenoside Rg1 group; E: High dose of ginsenoside Rg1 group.

图 2　各组小鼠的游泳路线图

Fig. 2　Swimming route maps of mice in various groups

2.3　各组小鼠血清中 BUN、LA、MDA、IL-6 和 TNF-α 水平及 LDH 和 SOD 活性　与对照组比较，模型组小鼠血清中 BUN、LA、MDA、IL-6 和 TNF-α 水平明显升高（$P<0.01$），LDH 活性明显升高（$P<0.01$），SOD 活性明显降低（$P<0.01$）；与模型组比较，抑制剂组和低及高剂量人参皂苷 Rg1 组小鼠血清中 BUN、LA、MDA、IL-6 和 TNF-α 水平明显降低（$P<0.01$），LDH 活性明显降低（$P<0.01$），SOD 活性明显升高（$P<0.01$）。见表 1 和 2。

表 1　各组小鼠血清中 BUN 和 LA 水平及 LDH 活性

Tab. 1　Levels of BUN and LA and activities of LDH in serum of mice in various groups　($n=8, \bar{x}\pm s$)

Group	BUN [c_B/(mmol·L^{-1})]	LA [c_B/(mmol·L^{-1})]	LDH [λ_B/(U·L^{-1})]
Control	6.21±0.55	5.36±0.71	535.66±50.43
Model	9.86±0.79*	8.86±0.85*	911.48±76.78*
Inhibitor	6.92±0.72△	7.04±0.59△	630.56±59.57△
Ginsenoside Rg1			
Low dose	6.75±0.73△	6.70±0.65△	599.12±54.81△
High dose	6.42±0.71△	5.78±0.68△	561.49±50.21△

*$P<0.01$ compared with control group; △$P<0.01$ compared with model group.

2.4　各组小鼠海马组织 CA1 区锥体神经元形态表现　与对照组比较，模型组小鼠海马组织 CA1 区中锥体神经元排列疏松，部分神经元呈三角形，核固缩，胞质浓染，少数神经元脱失，呈明显缺氧性神经元损伤；与模型组比较，抑制剂组、低和高剂量人参皂苷 Rg1 组小鼠海马组织 CA1 区中缺氧

表2　各组小鼠血清中MDA、TNF-α和IL-6水平及SOD活性

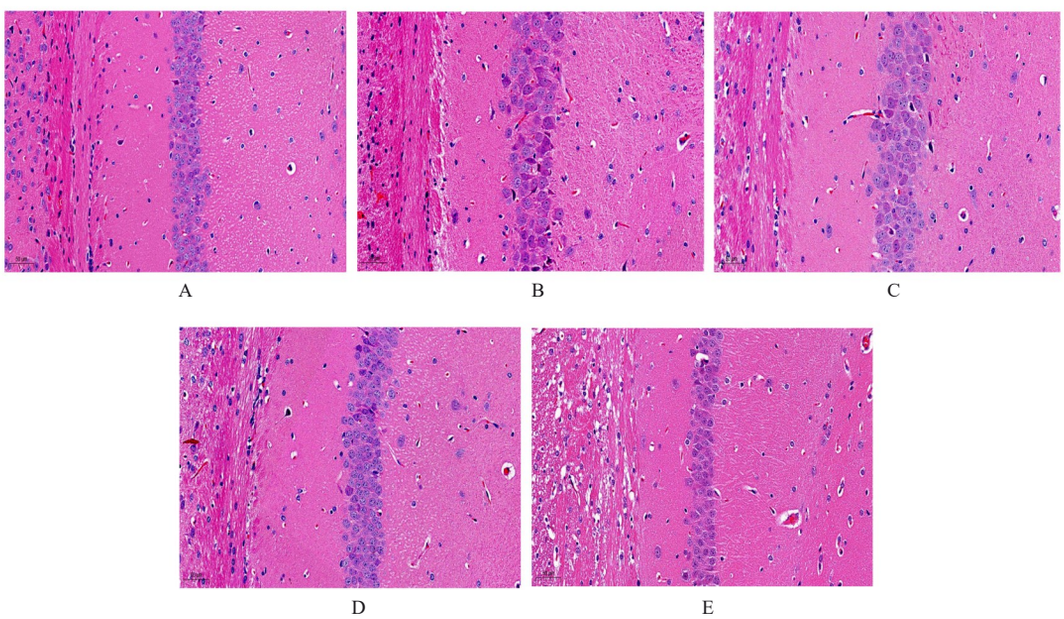
Tab. 2　Levels of MDA, TNF-α and of IL-6 and activities of SOD in serum of mice in various groups ($n=8, \bar{x}\pm s$)

Group	MDA [c_B/(mmol·L^{-1})]	TNF-α [ρ_B/(ng·L^{-1})]	IL-6 [ρ_B/(ng·L^{-1})]	SOD [λ_B/(U·L^{-1})]
Control	30.61±1.82	103.54±7.43	165.66±12.43	110.61±8.82
Model	64.47±3.04*	251.78±16.84*	336.74±22.84*	54.47±5.04*
Inhibitor	41.27±2.40△	164.12±10.04△	221.41±14.04△	90.27±7.40△
Ginsenoside Rg1				
Low dose	37.17±2.24△	133.21±9.21△	199.45±13.21△	94.17±8.24△
High dose	33.65±2.25△	110.84±8.99△	171.98±12.99△	102.65±7.24△

*$P<0.01$ compared with control group; △$P<0.01$ compared with model group.

性神经元损伤表现有效改善。见图3。

2.5　各组小鼠海马组织CA1区中ROS水平　与对照组比较，模型组小鼠海马组织CA1区中ROS水平明显升高（$P<0.01$）；与模型组比较，抑制剂组、低和高剂量人参皂苷Rg1组小鼠海马组织CA1区中ROS水平明显降低（$P<0.01$）。见图4和5。



A: Control group; B: Model group; C: Inhibitor group; D: Low dose of ginsenoside Rg1 group; E: High dose of ginsenoside Rg1 group.

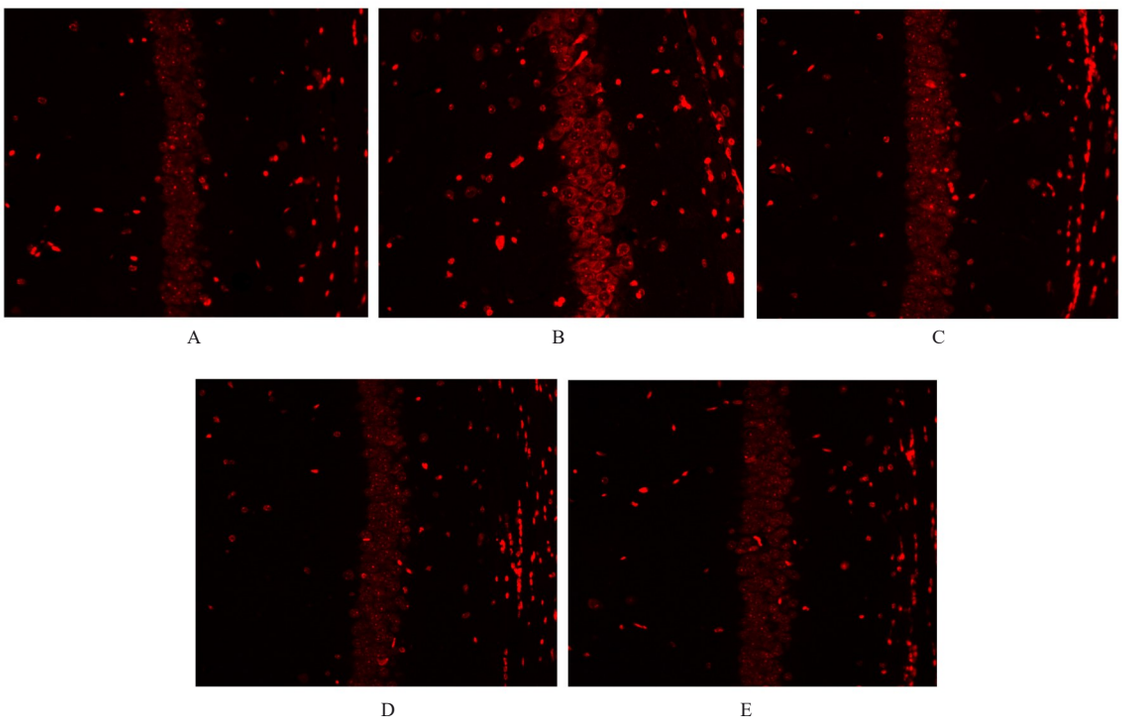
图3　各组小鼠海马组织CA1区中锥体神经元形态表现(HE，×200)

Fig. 3　Morphology of pyramidal neurons in CA1 area of hippocampus tissue of mice in various groups (HE, ×200)

2.6　各组小鼠海马组织中钙蛋白酶1、TNF-α和IL-6蛋白表达水平　与对照组比较，模型组小鼠海马组织中钙蛋白酶1、TNF-α和IL-6蛋白表达水平明显升高（$P<0.01$）；与模型组比较，抑制剂组、低和高剂量人参皂苷Rg1组小鼠海马组织中钙蛋白酶1、TNF-α和IL-6蛋白表达水平明显降低（$P<0.01$）。见图6。

3　讨　论

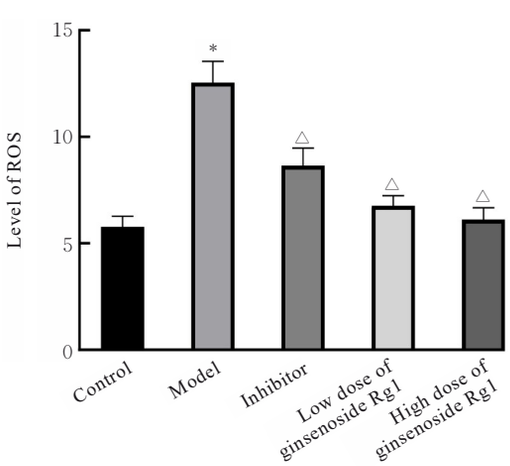
OSA是临床常见疾病，已成为全球最普遍的慢性疾病之一[10]。OSA可引起多脏器功能损伤，其中OSA引起的脑损伤是研究热点[11]。长期CIH可导致大脑神经退行性病变，累及学习记忆、注意力和运动的神经递质系统[12]。多种病理生理过程参与CIH导致的大脑神经损害，包括氧化应激、炎症、环境和基因改变[13]。研究[14]显示：CIH引起脑组织中炎症因子IL-6和TNF-α表达升高。此外，CIH会导致线粒体氧化磷酸化超负荷，刺激ROS过度生成，使线粒体功能受损，引起神经元氧化应激损伤[15]。研究[16]显示：CIH模型小鼠SpO_2为60%～85%。在缺氧情况下，血清中BUN、LDH



A：Control group；B：Model group；C：Inhibitor group；D：Low dose of ginsenoside Rg1 group；E：High dose of ginsenoside Rg1 group.

图 4　各组小鼠海马组织 CA1 区 ROS 表达情况（DHE，×200）

Fig. 4　Expressions of ROS in CA1 area of hippocampus tissue of mice in various groups（DHE，×200）



*P<0.01 compared with control group；△P<0.01 compared with model group.

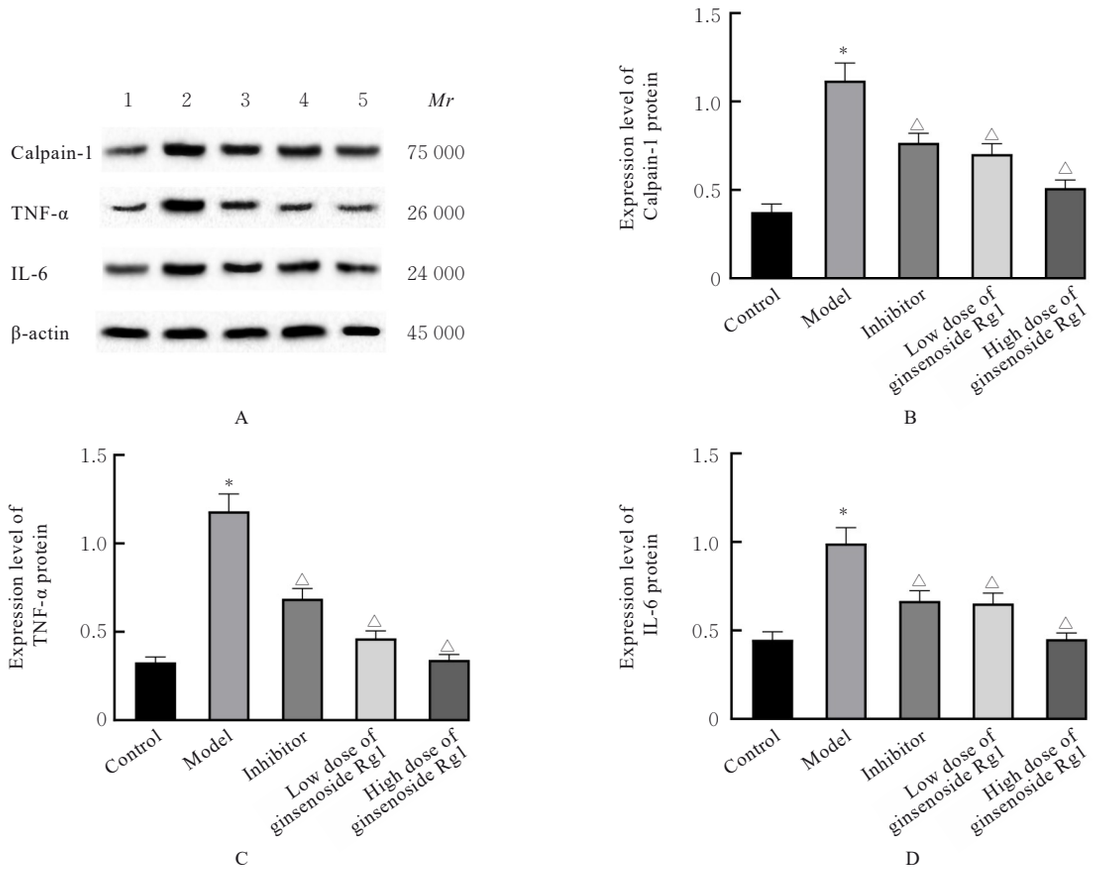
图 5　各组小鼠海马 CA1 区中 ROS 水平

Fig. 5　Levels of ROS in CA1 area of hippocampus tissue of mice in various groups

和 LA 等生化指标升高[17]。本研究结果显示：CIH 模型小鼠 SpO_2 为 60%～85%，血清中 BUN、LDH 和 LA 等生化指标升高，学习记忆能力降低，提示模型制备成功；10 和 20 mg·kg^{-1}人参皂苷 Rg1 可改善小鼠 SpO_2 和学习记忆能力，降低 BUN、LDH 和 LA，抑制 CIH 诱导的氧化应激和炎症反应，说明人参皂苷 Rg1 对 CIH 诱导的脑损伤有一定的保护作用。

钙蛋白酶是由钙激活的非溶酶体中性半胱氨酸蛋白酶[18]，在大脑中表达的最具特征的亚型是钙蛋白酶 1 和钙蛋白酶 2，其中以钙蛋白酶 1 表达最普遍[19]。研究[20]显示：在缺氧条件下，钙蛋白酶 1 失调使机体细胞中 Ca^{2+} 超载，激活许多依赖 Ca^{2+} 的蛋白酶，导致急性和慢性神经退行性病变，此外，激活钙蛋白酶 1 表达可使 IL-6 和 TNF-α 表达水平升高，加剧氧化应激反应，从而导致继发性神经系统损伤[21]。钙蛋白酶 1 在黄芪甲苷对脑损伤的保护中发挥作用[6]。本研究结果显示：CIH 诱导脑损伤后钙蛋白酶 1 表达水平升高。发生 CIH 时给予人参皂苷 Rg1 或钙蛋白酶 1 抑制剂处理，不但可以抑制 CIH 诱导的脑损伤炎症反应和氧化应激，还可以降低钙蛋白酶 1 表达水平，说明人参皂苷 Rg1 对 CIH 诱导的脑损伤的保护作用可能与抑制钙蛋白酶 1 通路有关。

人参皂苷 Rg1 为我国传统名贵中药人参的主要活性成分之一[22]。研究[23]显示：人参皂苷 Rg1 具有一定的机体保护作用，但其具体机制尚不明确。本研究在动物水平上探讨了人参皂苷 Rg1 对 CIH 诱



Lane 1: Control group; Lane 2: Model group; Lane 3: Inhibitor group; Lane 4: Low dose of ginsenoside Rg1 group; Lane 5: High dose of ginsenoside Rg1 group. *$P<0.01$ compared with control group; △$P<0.01$ compared with model group.

图6　各组小鼠海马组织中Calpain-1、TNF-α和IL-6蛋白表达电泳图(A)和直条图(B～D)

Fig. 6　Electrophoregram (A) and histograms (B－D) of expressions of Calpain-1, TNF-α, and IL-6 proteins in hippocampus tissue of mice in various groups

导脑损伤的保护作用及其潜在机制，进一步明确了人参皂苷Rg1对脑损伤的保护作用。

综上所述，人参皂苷Rg1可改善CIH诱导脑损伤小鼠的学习记忆能力、炎症反应和氧化应激，其潜在分子机制可能与抑制钙蛋白酶1通路有关。该结论为人参皂苷Rg1在脑损伤相关疾病的临床应用提供了依据。

利益冲突声明：

所有作者声明不存在利益冲突。

作者贡献声明：

孟岩参与论文选题、实验设计、实验操作、数据收集、数据分析、统计学分析和论文撰写，王洪新和杨育红参与论文选题、实验设计和论文修改。

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