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鼠尾草酸调控Keap1-Nrf2通路对青光眼大鼠视神经损伤的影响

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摘要 该研究探讨鼠尾草酸(CA)对青光眼大鼠视神经损伤及Keap1-Nrf2通路的影响。首先, 构建青光眼大鼠模型; 将造模成功大鼠随机分为模型组(Model组), 鼠尾草酸低、高剂量处理组(CA-L、CA-H组), 鼠尾草酸高剂量处理+Nrf2抑制剂组(CA-H+ML385), 每组18只, 另取18只健康大鼠作为对照组(Control组); 检测各组大鼠眼压; ELISA检测视网膜氧化应激水平; HE染色观察视网膜病理损伤; 荧光金逆行标记大鼠视网膜神经节细胞(RGCs); 视网膜铺片观察RGCs细胞并计数; TUNEL染色观察RGCs细胞凋亡; Western blot检测视网膜组织Keap1-Nrf2通路及凋亡相关蛋白表达情况。结果显示, Model组较Control组大鼠眼压, ROS、MDA水平, RGCs凋亡率, Bax/Bcl-2、Cleaved-caspase-3/caspase-3值, Keap1表达水平升高, 神经纤维层厚度, RGCs存活数目, SOD活性, Nrf2、HO-1表达水平降低(P<0.05); CA-L、CA-H组较Model组大鼠眼压, ROS、MDA水平, RGCs凋亡率, Bax/Bcl-2、Cleaved-caspase-3/caspase-3值, Keap1表达水平降低, 神经纤维层厚度, RGCs存活数目, SOD活性, Nrf2、HO-1表达水平升高(P<0.05); CA-H+ML385组较CA-H组大鼠眼压, ROS、MDA水平, RGCs凋亡率, Bax/Bcl-2、Cleaved-caspase-3/caspase-3值, Keap1表达水平升高, 神经纤维层厚度, RGCs存活数目, SOD活性, Nrf2、HO-1表达水平降低(P<0.05)。总之, 鼠尾草酸可减轻青光眼大鼠神经损伤, 与调节Keap1-Nrf2通路有关。

关键词 鼠尾草酸; Keap1-Nrf2通路; 青光眼; 视神经损伤

The Impacts of Carnosic Acid on Optic Nerve Injury in Glaucoma Rats by Adjusting Keap1-Nrf2 Pathway

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Abstract This study explores the impacts of CA (carnosic acid) on optic nerve injury in glaucomatous rats and the Keap1-Nrf2 pathway. Firstly, establish a rat model of glaucoma, and successfully modeled rats were stochastically assigned into a Model group, CA-L, CA-H groups (low and high-dose carnosic acid treatment groups), and CA-H+ML385 group (high-dose carnosic acid treatment+Nrf2 inhibitor), each with 18 rats. Additionally, 18 healthy rats were designated as Control group. The intraocular pressure of rats in each group was measured. ELISA

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was used to detect retinal oxidative stress levels. HE staining was used to observe retinal pathological damage. The fluorescence gold retrograde method was used to label rat RGCs (retinal ganglion cells). Retinal capture was performed to observe and count RGCs cells. TUNEL staining was used to observe apoptosis of RGCs cells. Western blot was performed to detect the Keap1-Nrf2 pathway and apoptosis related proteins in retinal tissue. The results showed that compared with the Control group, the levels of intraocular pressure, ROS, MDA, as well as the apoptosis rate of RGCs, the ratios of Bax/Bcl-2, Cleaved-caspase-3/caspase-3, and Keap1 expression levels in the Model group were all increased, while the thickness of the nerve fiber layer, the number of surviving RGCs, the activity of SOD, the expressions levels of Nrf2 and HO-1 were all decreased (P<0.05). Compared with the Model group, the levels of intraocular pressure, ROS, MDA, as well as the apoptosis rate of RGCs, the ratios of Bax/Bcl-2, Cleaved-caspase-3/caspase-3, and Keap1 expression levels in the CA-L and CA-H groups decreased, while the thickness of the nerve fiber layer, the number of surviving RGCs, the activity of SOD, and the expressions levels of Nrf2 and HO-1 increased (P<0.05). Compared with the CA-H group, the levels of intraocular pressure, ROS, MDA, as well as the apoptosis rate of RGCs, the ratios of Bax/Bcl-2, Cleaved-caspase-3/caspase-3, and Keap1 expression levels were all increased in the CA-H+ML385 group, while the thickness of the nerve fiber layer, the number of surviving RGCs, the activity of SOD, the expression levels of Nrf2 and HO-1 were all decreased (P<0.05). In conclusion, carnosic acid can improve nerve damage in glaucoma rats, which is related to adjusting Keap1-Nrf2 pathway.

Keywords carnosic acid; Keap1-Nrf2 pathway; glaucoma; optic nerve injury

青光眼是一种慢性进行性第二大致盲视神经疾病, 而且其具有不可逆性, 主要表现为视网膜神经节细胞(retinal ganglion cells, RGCs)缺失、视神经萎缩、视野缺损等, 多发生于60岁以上的人、已经被诊断患有青光眼的人的家庭成员、糖尿病患者、类固醇使用者及患有高血压、高度近视、中央角膜厚度<5 mm和患眼外伤的人[1]。近年来青光眼发病率随着全球人口老龄化加剧不断升高, 预计2040年将有1.118亿人患有青光眼, 其严重影响患者的视力健康和生活质量, 并给社会带来沉重负担[2]。然而青光眼早期症状不明显, 甚至碍于筛查的局限性, 许多病例直到晚期仍未得到诊断, 导致治疗效率不高。而眼压作为治疗青光眼唯一可改变的危险因素, 降低眼内压可有效降低青光眼的视力丧失率, 目前主要通过手术、局部用药、激光治疗等治疗方式降低眼内压, 以缓解青光眼症状, 但治疗有限, 仍需探索新的治疗方式与药物[3]。鼠尾草酸是广泛存在于迷迭香等唇科类植物中的一种酚萜类化合物, 对于治疗神经系统、心血管、视网膜等组织器官的病变具有疗效。其中鼠尾草酸可减少RGCs凋亡数量, 增加RGCs存活数量, 抑制急性高眼压大鼠RGCs凋亡并对其发挥保护作用[4]。Keap1-Nrf2通路是经典的抗氧化应激通路, 研究发现, 抑制Keap1-Nrf2通路可升高术后视网膜神经节细胞的存活率, 改善视觉功能, 对青光眼表现出神经保护作用[5]。本研究主要探究鼠尾草酸能否通过调控Keap1-Nrf2通路减轻青光眼大鼠视神经损伤, 以期为青光眼治疗寻求新策略。

1 材料与方法

1.1 主要材料

体质量(200±20) g SPF级7~8周龄雄性SD大鼠购自武汉市万千佳兴生物科技有限公司[SCXK(鄂)2021-0011], 本研究已获得武汉市万千佳兴生物科技有限公司动物伦理委员会审批(批号: 202403057); ML385(HPLC≥99%, 货号: M304758)购自上海阿拉丁生化科技股份有限公司; Bax(货号: FNab00809)、Bcl-2抗体(货号: FNab00839)购自武汉菲恩生物科技有限公司; Cleaved-caspase-3(货号: WL01992)、caspase-3抗体(货号: WL04004)购自沈阳万类生物科技有限公司; Keap1(货号: DL95130A)、Nrf2(货号: DL90797A)、HO-1抗体(货号: DL96868A)购自无锡市东林科技发展有限责任公司。

1.2 方法

1.2.1 青光眼大鼠模型构建 所有大鼠适应性喂养7天, 戊巴比妥钠麻醉大鼠后将其俯卧位固定于手术台上, 然后用0.5%盐酸丙美卡因滴眼麻醉大鼠右眼, 并对其眼部进行消毒, 眼科显微镜下钝性分离球结膜及筋膜, 然后暴露并选择3支上巩膜静脉用手术

烧灼器轻轻灼烧, 发现远角膜端血管血流消失, 近角膜端血管扩张充盈, 表示烙灼成功, 术后7天检测大鼠右眼眼压, 若其≥21 mmHg或高于左眼眼压5 mmHg, 表示造模成功[6]。对照组同步操作, 只是上巩膜静脉未进行烧灼处理。

1.2.2 分组与处理 将造模成功的大鼠随机分为模型组(Model组), 鼠尾草酸低、高剂量处理组(CA-L、CA-H组), 鼠尾草酸高剂量处理+Nrf2抑制剂组(CA-H+ML385), 每组18只, 另取18只健康大鼠作为对照组(Control组); CA-L、CA-H组[4]: 术后每天分别腹腔注射12.5、25.0 mg/kg鼠尾草酸及玻璃体腔内注射0.5 mg/kg生理盐水; CA-H+ML385[7]: 术后每天分别腹腔注射25 mg/kg鼠尾草酸及玻璃体腔内注射1 μL(0.1 mg/μL) Nrf2抑制剂ML385(用专用的注射针头, 在睫状体平坦部, 角巩膜缘后大致3~4 mm进针, 将药物注射至玻璃体腔); Model组与Control组分别腹腔注射及玻璃体腔内注射等量二甲基亚砜; 连续给药7天。

1.2.3 眼压测量 给药前及末次给药后2 h、7天、14天, 麻醉大鼠并将其固定于手术台, 然后用0.5%盐酸丙美卡因滴眼麻醉大鼠右眼, 使用眼压计(icare)测量眼压。

1.2.4 氧化应激水平检测 眼压测量结束, 各组选择12只大鼠戊巴比妥纳麻醉并通过心脏灌注4%多聚甲醛将其处死, 取右眼眼球并分离视网膜组织, 然后任选各组其中6个制成匀浆, 4 ℃、3 000 r/min离心15 min, 取上清液, ELISA试剂盒检测视网膜组织ROS、MDA水平及SOD活性, 剩余视网膜组织于液氮中保存。

1.2.5 视网膜病理损伤观察 剩余视网膜组织各组任选6个, 4%多聚甲醛4 ℃固定12 h, 固定后, 依次通过70%、80%、90%、100%梯度酒精脱水, 再经二甲苯透明, 行石蜡包埋, 切成厚约5 μm的切片, 60 ℃烤片20 min。随后, 将切片依次浸泡于脱蜡剂A、B、C各15 min, 再经100%酒精、95%酒精、90%酒精复水, 结束后用自来水冲洗5 min。将切片浸入苏木精染液中染色2~3 min, 自来水冲洗2次。随后, 用1%盐酸酒精分化5 s, 自来水返蓝10 min, 甩干水分。使用伊红染液染色1 min, 自来水快速冲洗甩干, 并经90%、95%、100%酒精脱水, 二甲苯透明后, 采用中性树脂封片。于封片结束, 将切片置于光学显微镜下观察分析, 并用视盘周围环扫方法检测视网膜神经纤维层厚度。

1.2.6 荧光金逆行标记大鼠RGCs 各组剩余6只大鼠经戊巴比妥纳麻醉后将其固定于脑立体定位仪上, 沿颅顶正中线切开头皮, 暴露颅骨矢状缝和人字缝, 定位Bregma点并以其为零点, 在其后方4.3、6.3 mm处分别旁开4.4、1.4 mm处用牙钻钻开骨孔, 暴露右侧外侧膝状体及双侧上丘, 然后微玻管分别在深度4.5、3.5 mm处注入0.5 μL 3%荧光金, 滞留10 min后, 分层缝合伤口, 待苏醒后自由饮食饮水。

1.2.7 视网膜铺片观察RGCs并计数 将1.2.6大鼠麻醉处死, 摘除右眼球, 分离视网膜, 浸入4%多聚甲醛4 ℃固定0.5~1 h后平铺于载玻片, 甘油PBS封片后, 置于荧光显微镜下观察, 其中将视盘分成相等的鼻上、鼻下、颞上、颞下4个象限并对每个象限距视盘中心2 mm处进行拍照, 并分析RGCs存活数量。

1.2.8 TUNEL染色检测RGCs凋亡 将1.2.5剩余视网膜石蜡切片脱蜡至水, 先加入蛋白酶K室温孵育15~30 min透化, 再进行3% H_2O_2室温避光灭活, 然后在室温下加入TUNEL试剂避光37 ℃孵育60 min, 再进行DAPI细胞核染色, 然后进行显色处理后苏木精复染细胞核, 荧光显微镜下观察分析RGCs凋亡情况。

1.2.9 Western blot检测视网膜组织Keap1-Nrf2通路及凋亡相关蛋白表达情况 蛋白裂解液提取1.2.4视网膜组织匀浆总蛋白并定量, 然后沸水浴变性, SDS-PAGE电泳分离后, 将蛋白湿转PVDF膜并用血清封闭, 然后在低温下加入一抗Keap1(1:500)、Nrf2(1:500)、HO-1(1:500)、Bax(1:500)、Bcl-2(1:500)、Cleaved-caspase-3(1:500)、caspase-3(1:500) 4 ℃孵育过夜, 次日转入室温下与二抗(1:2 000)室温孵育1 h, 然后进行可视化处理, 收集图像并用ImageJ软件分析计算相对表达量。

1.3 统计学方法

采用SPSS 25.0进行统计分析, 计量资料符合正态分布以$\bar{x}\pm s$描述, 多组间比较采用单因素方差分析, 组间两两比较采用SNK-q检验。以$P<0.05$时表示差异有统计学意义。

2 结果

2.1 鼠尾草酸对青光眼大鼠眼压的影响

末次给药后, Model组较Control组大鼠眼压升高($P<0.05$); CA-L、CA-H组较Model组大鼠眼压降低($P<0.05$); CA-H+ML385组较CA-H组大鼠眼压升高($P<0.05$); 见表1。

表1 各组大鼠眼压比较

Table 1 Comparison of intraocular pressure among different groups of rats

组别 Group	眼压/mmHg Intraocular pressure /mmHg				
	造模前 Before the model was created	给药前 Before administration	末次给药后2 h 2 h after the last administration	末次给药后7天 7 days after the last administration	末次给药后14天 14 days after the last administration
Control	16.02±1.67	16.05±1.73	16.11±1.78	16.08±1.76	16.12±1.80
Model	16.04±1.70	33.14±3.58*	32.27±3.35*	32.59±3.41*	32.87±3.43*
CA-L	16.08±1.77	32.96±3.39*	24.58±2.52$^{\#}$	24.76±2.61$^{\#}$	24.98±2.63$^{\#}$
CA-H	16.05±1.72	33.05±3.46*	17.46±1.80$^{\#\&}$	17.65±1.84$^{\#\&}$	17.81±1.88$^{\#\&}$
CA-H+ML385	16.07±1.75	32.09±3.31*	24.19±2.49$^{@}$	24.37±2.55$^{@}$	24.66±2.62$^{@}$

$\bar{x}\pm s$, n=18。*P<0.05, 与Control组比较; $^{\#}P$<0.05, 与Model组比较; $^{\&}P$<0.05, 与CA-L组比较; $^{@}P$<0.05, 与CA-H组比较。

$\bar{x}\pm s$, n=18. *P<0.05 compared with the Control group; $^{\#}P$<0.05 compared with the Model group; $^{\&}P$<0.05 compared with the CA-L group; $^{@}P$<0.05 compared with the CA-H group.

表2 各组大鼠氧化应激水平比较

Table 2 Comparison of oxidative stress levels in each group of rats

组别 Group	ROS /U·mL^{-1}	MDA /nmol·mL^{-1}	SOD /U·mL^{-1}
Control	286.41±31.54	4.06±0.52	124.75±13.66
Model	492.57±53.86*	9.23±1.17*	60.58±7.12*
CA-L	405.63±42.91$^{\#}$	6.74±0.84$^{\#}$	86.17±9.83$^{\#}$
CA-H	311.29±33.05$^{\#\&}$	4.31±0.60$^{\#\&}$	120.94±12.95$^{\#\&}$
CA-H+ML385	390.28±40.76$^{\#\&}$	6.65±0.73$^{\#\&}$	91.63±10.39$^{\#\&}$

$\bar{x}\pm s$, n=18。*P<0.05, 与Control组比较; $^{\#}P$<0.05, 与Model组比较; $^{\&}P$<0.05, 与CA-L组比较; $^{@}P$<0.05, 与CA-H组比较。

$\bar{x}\pm s$, n=18. *P<0.05 compared with the Control group; $^{\#}P$<0.05 compared with the Model group; $^{\&}P$<0.05 compared with the CA-L group; $^{@}P$<0.05 compared with the CA-H group.

2.2 鼠尾草酸对青光眼大鼠视网膜氧化应激水平的影响

Model组较Control组ROS、MDA水平升高, SOD活性降低(P<0.05); CA-L、CA-H组较Model组ROS、MDA水平降低, SOD活性升高(P<0.05); CA-H+ML385组较CA-H组ROS、MDA水平升高, SOD活性降低(P<0.05); 见表2。

2.3 鼠尾草酸对青光眼大鼠视网膜病理损伤的影响

Model组较Control组神经纤维层厚度降低(P<0.05); CA-L、CA-H组较Model组神经纤维层厚度升高(P<0.05); CA-H+ML385组较CA-H组神经纤维层厚度降低(P<0.05); 见图1和表3。

2.4 鼠尾草酸对青光眼大鼠视网膜RGCs数量的影响

Model组较Control组RGCs存活数目减少(P<0.05); CA-L、CA-H组较Model组RGCs存活数目增多(P<0.05); CA-H+ML385组较CA-H组RGCs存活数目减少(P<0.05); 见图2和表3。

2.5 鼠尾草酸对青光眼大鼠视网膜RGCs凋亡的影响

Model组较Control组大鼠RGCs凋亡率及Bax/Bcl-2、Cleaved-caspase-3/caspase-3值升高(P<0.05); CA-L、CA-H组较Model组大鼠RGCs凋亡率及Bax/Bcl-2、Cleaved-caspase-3/caspase-3值降低(P<0.05); CA-H+ML385组较CA-H组大鼠RGCs凋亡率及Bax/Bcl-2、Cleaved-caspase-3/caspase-3值升高(P<0.05); 见图3、图4和表4。

2.6 鼠尾草酸对Keap1-Nrf2通路的影响

Model组较Control组Keap1表达水平升高, Nrf2、HO-1表达水平降低(P<0.05); CA-L、CA-H组较Model组Keap1表达水平降低, Nrf2、HO-1表达水平升高(P<0.05); CA-H+ML385组较CA-H组Keap1表达水平升高, Nrf2、HO-1表达水平降低(P<0.05); 见图5和表5。

3 讨论

青光眼是一种不可逆的慢性进行性神经退行

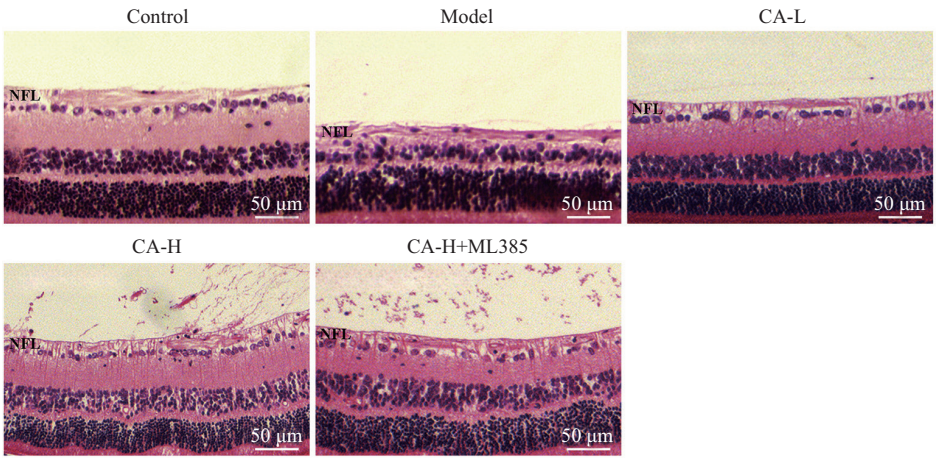


图1 HE染色观察视网膜组织病理形态

Fig.1 HE staining observation of retinal tissue pathological morphology

表3 各组大鼠神经纤维层厚度及RGCs存活数目比较

Table 3 Comparison of nerve fiber layer thickness and the number of surviving RGCs in each group of rats

组别 Group	神经纤维层厚度/μm Thickness of the nerve fiber layer /μm	RGCs存活数目 The number of surviving retinal ganglion cells
Control	13.85±1.42	2 415.63±255.34
Model	6.18±0.67*	996.48±107.62*
CA-L	8.82±0.93$^{\#}$	1 579.43±161.27$^{\#}$
CA-H	12.79±1.31$^{\#\&}$	2 144.57±220.46$^{\#\&}$
CA-H+ML385	8.96±0.97$^{@}$	1 693.28±172.19$^{@}$

$\bar{x}\pm s$, n=6。*P<0.05, 与Control组比较; $^{\#}P$<0.05, 与Model组比较; $^{\&}P$<0.05, 与CA-L组比较; $^{@}P$<0.05, 与CA-H组比较。

$\bar{x}\pm s$, n=6. *P<0.05 compared with the Control group; $^{\#}P$<0.05 compared with the Model group; $^{\&}P$<0.05 compared with the CA-L group; $^{@}P$<0.05 compared with the CA-H group.

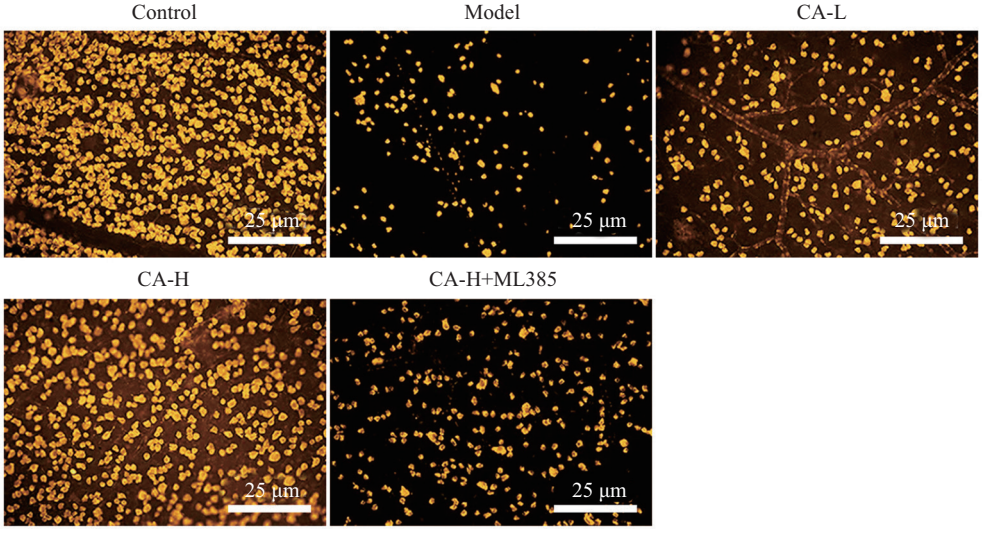
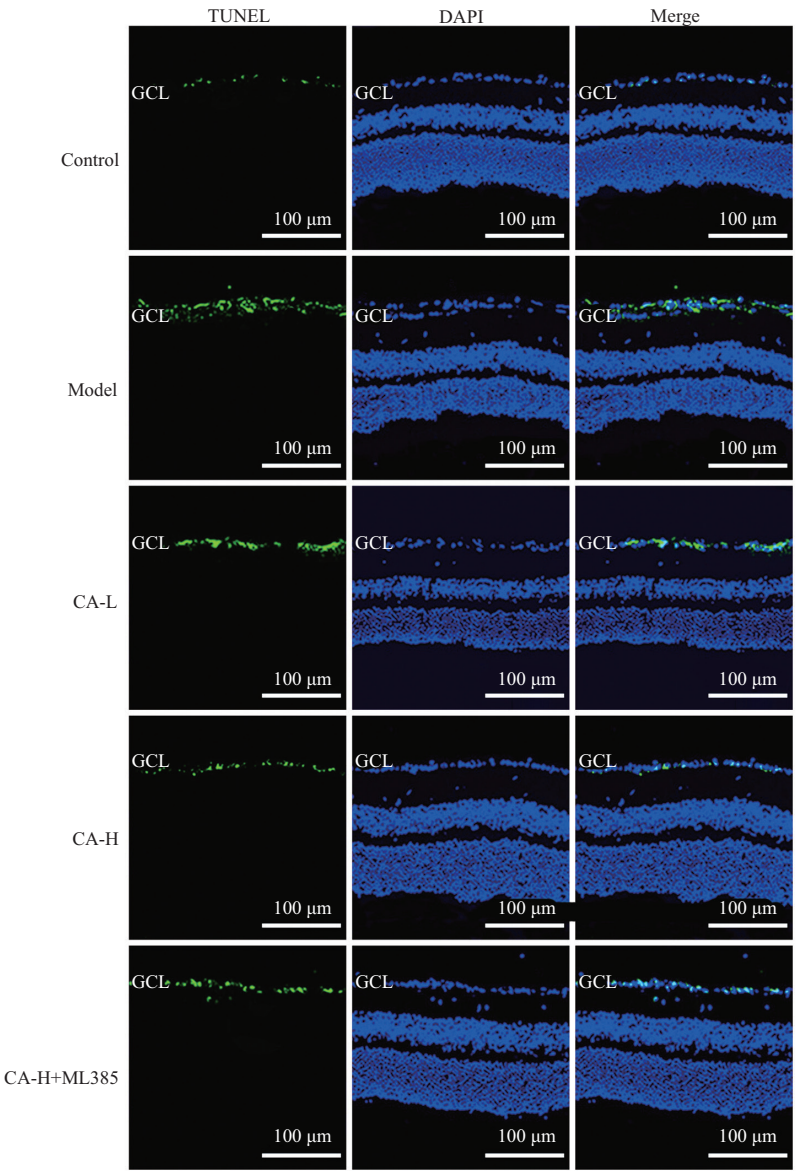


图2 荧光金逆行标记大鼠RGCs

Fig.2 Fluorescent gold retrograde labeling of rat retinal ganglion cells

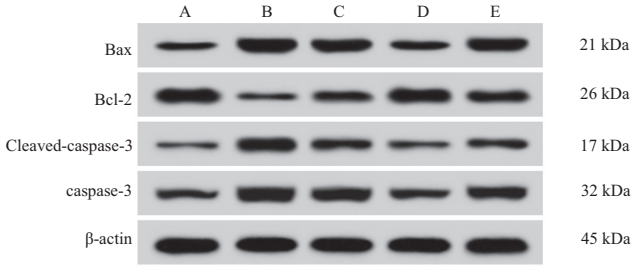
性疾病, 其可受眼内压、高龄、青光眼家族史等因素影响, 引发视网膜发生炎症、氧化应激、RGCs不可逆凋亡等病理过程, 促进青光眼发生发展, 进而造成患者视力下降甚至永久失明[8]。目前主要通过眼压测量、视野检查、眼底镜检查等进行诊断然后采取手术、药物、激光等方式以降低眼内压, 减少



GCL: 视网膜神经节细胞层。
GCL: ganglion cell layer.

图3 TUNEL染色观察视网膜RGCs凋亡

Fig.3 Observation of retinal RGCs apoptosis using TUNEL staining



A: Control组; B: Model组; C: CA-L组; D: CA-H组; E: CA-H+ML385组。
A: Control group; B: Model group; C: CA-L group; D: CA-H group; E: CA-H+ML385 group.

图4 Western blot检测视网膜组织Bax、Bcl-2、Cleaved-caspase-3、caspase-3表达情况

Fig.4 Western blot analysis of Bax, Bcl-2, Cleaved-caspase-3 and caspase-3 expressions in retinal tissue

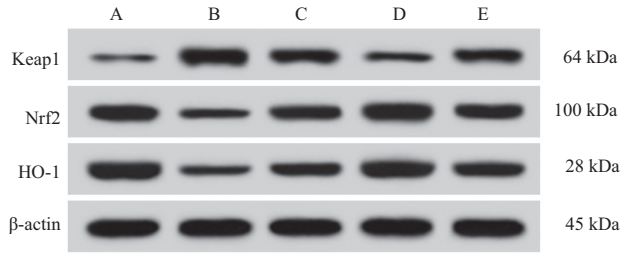
表4 各组大鼠视网膜RGCs凋亡情况比较

Table 4 Comparison of retinal RGCs apoptosis in each group of rats

组别 Group	凋亡率/% Apoptosis rate /%	Bax/Bcl-2	Cleaved-caspase-3/caspase-3
Control	5.83±0.61	0.44±0.05	0.53±0.06
Model	28.96±2.95*	2.97±0.32*	0.92±0.10*
CA-L	19.44±2.03$^{\#}$	1.33±0.15$^{\#}$	0.72±0.08$^{\#}$
CA-H	9.85±1.06$^{\#\&}$	0.56±0.07$^{\#\&}$	0.51±0.06$^{\#\&}$
CA-H+ML385	18.62±1.92$^{@}$	1.17±0.13$^{@}$	0.71±0.08$^{@}$

$\bar{x}\pm s$, n=6。*P<0.05, 与Control组比较; $^{\#}P$<0.05, 与Model组比较; $^{\&}P$<0.05, 与CA-L组比较; $^{@}P$<0.05, 与CA-H组比较。

$\bar{x}\pm s$, n=6. *P<0.05 compared with the Control group; $^{\#}P$<0.05 compared with the Model group; $^{\&}P$<0.05 compared with the CA-L group; $^{@}P$<0.05 compared with the CA-H group.



A: Control组; B: Model组; C: CA-L组; D: CA-H组; E: CA-H+ML385组。

A: Control group; B: Model group; C: CA-L group; D: CA-H group; E: CA-H+ML385 group.

图5 Western blot检测视网膜组织Keap1、Nrf2、HO-1表达情况

Fig.5 Western blot analysis of Keap1, Nrf2 and HO-1 expressions in retinal tissue

表5 各组大鼠视网膜Keap1-Nrf2通路相关蛋白表达比较

Table 5 Comparison of Keap1-Nrf2 pathway-related protein expressions in the retinas of each group of rats

组别 Group	Keap1	Nrf2	HO-1
Control	0.22±0.03	0.89±0.10	0.80±0.09
Model	0.82±0.09*	0.36±0.05*	0.27±0.04*
CA-L	0.58±0.07$^{\#}$	0.57±0.07$^{\#}$	0.48±0.06$^{\#}$
CA-H	0.31±0.04$^{\#\&}$	0.83±0.09$^{\#\&}$	0.74±0.08$^{\#\&}$
CA-H+ML385	0.53±0.06$^{@}$	0.60±0.07$^{@}$	0.52±0.06$^{@}$

$\bar{x}\pm s$, n=6。*P<0.05, 与Control组比较; $^{\#}P$<0.05, 与Model组比较; $^{\&}P$<0.05, 与CA-L组比较; $^{@}P$<0.05, 与CA-H组比较。

$\bar{x}\pm s$, n=6. *P<0.05 compared with the Control group; $^{\#}P$<0.05 compared with the Model group; $^{\&}P$<0.05 compared with the CA-L group; $^{@}P$<0.05 compared with the CA-H group.

RGCs不可逆凋亡, 进而延缓视野缺损进展[9]。鼠尾草酸作为提取自迷迭香等唇科类植物的一种酚萜类化合物, 其具有抗氧化、抗凋亡等多种生理活性, 可维持机体氧化还原体系的稳定, 减轻神经心血管疾病、衰老、视网膜病变等多种氧化损伤[10]。而且对鼠尾草酸进行药代动力学研究, 发现健康志愿者口服鼠尾草酸后, 通过高效液相色谱紫外检测法分析人血浆中鼠尾草酸, 其回收率为91.7%, 具有高生物利用度[11]。本研究显示, 青光眼大鼠眼压异常高, 神经纤维层厚度及RGCs存活数目减少, 而鼠尾草酸治疗可降低大鼠眼压, 增加神经纤维层厚度及RGCs存活数目。另有研究显示, 鼠尾草酸可抑制毒死蜱诱导的小鼠眼组织氧化应激和炎症反应, 进而发挥神经保护作用[12]。推测鼠尾草酸可对青光眼大鼠发挥神经保护作用。

氧化应激反应是大鼠青光眼发生发展的重要病理过程。主要是当眼部受到病理刺激时, 视网膜组织可生成大量活性氧, 并抑制抗氧化活性酶SOD

等活性，促使剩余氧自由基氧化细胞内脂质、蛋白质等物质，生成大量脂质氧化物MDA损伤视网膜、视乳头、小梁网等，进而加剧眼内压的升高，发生氧化应激反应，促进RGCs凋亡，影响视觉神经通路，促进青光眼发生发展[13]。本研究显示，青光眼大鼠视网膜ROS、MDA水平升高，SOD活性降低，而鼠尾草酸治疗可降低ROS、MDA水平，提高SOD活性，说明鼠尾草酸治疗可通过抑制氧化应激减轻青光眼大鼠神经损伤。另有研究显示，谷氨酸可通过诱导ROS过量产生，加剧视网膜损伤及RGCs丢失，促进青光眼发生[14]。而提高SOD活性，降低MDA水平可抑制氧化应激反应，对青光眼大鼠及RGCs发挥保护作用[15-16]。

RGCs凋亡是青光眼的主要病理表现。而Bax、Bcl-2、Cleaved-caspase-3、caspase-3均是参与RGCs凋亡过程中的关键蛋白，其中促凋亡蛋白Bax与抑凋亡蛋白Bcl-2两者相互拮抗，Bax/Bcl-2值可以反映出细胞凋亡情况。另外caspase-3是细胞凋亡执行蛋白，其活化后可形成Cleaved-caspase-3促进细胞凋亡。本研究发现，青光眼大鼠视网膜组织中Bax/Bcl-2、Cleaved-caspase-3/caspase-3值均上调，而鼠尾草酸治疗可下调Bax/Bcl-2、Cleaved-caspase-3/caspase-3值，抑制RGCs凋亡，说明鼠尾草酸治疗可通过抑制RGCs凋亡减轻青光眼大鼠神经损伤。另有研究显示，降低Bax/Bcl-2值及caspase-3表达水平可减少视网膜神经元凋亡，减轻视网膜病理损伤，对青光眼大鼠发挥视网膜神经保护作用[17]。降低caspase-3和BAX表达水平，促进Bcl-2表达可减少RGCs损失，抑制氨酸诱导的视网膜神经节细胞层和双极细胞层厚度的降低，对青光眼小鼠发挥保护作用[18]。

青光眼、白内障等年龄相关性眼病发生发展与眼部氧化应激有重要关系，而Keap1-Nrf2通路作为经典抗氧化应激通路，在受到氧化刺激时，Keap1与Nrf2在细胞质中发生解离，随后Nrf2进入细胞核与抗氧化反应元件ARE结合，激活下游抗氧化蛋白基因表达，提高抗氧化酶HO-1、SOD等活性，发挥抗氧化反应，进而抑制RGCs凋亡，减轻氧化损伤[19]。本研究发现，青光眼大鼠视网膜组织中Keap1表达上调，而Nrf2、HO-1表达下调。研究显示，下调Keap1促进Nrf2/ARE激活可抑制氧化应激反应及RGCs凋亡，进而抑制青光眼视网膜变性发展[20]。激活Nrf2/ARE通路可延缓青光眼病理学的发作，对其发挥神经保护作用[21]。增加Nrf2表达量治疗RGCs可促进抗氧化基因的转录，增强抗氧化反应，进而延迟视力丧失和轴突变性的发作，抑制更早和更严重的青光眼病变[22]。推测抑制Keap1-Nrf2通路可减轻青光眼大鼠神经损伤。本研究还发现鼠尾草酸治疗可下调Keap1表达，上调Nrf2、HO-1表达。而且鼠尾草酸可通过激活Keap1-Nrf2途径发挥抗氧化、抗炎和神经保护作用，进而对新冠及阿尔茨海默病、帕金森病等其他慢性神经退行性疾病发挥治疗作用[23]。另外通过Nrf2抑制剂处理，发现Keap1表达上调，而Nrf2、HO-1表达下调。这表明鼠尾草酸治疗可抑制Keap1-Nrf2通路发挥抗氧化作用，进而减轻青光眼大鼠神经损伤。

综上，鼠尾草酸可减轻青光眼大鼠神经损伤，与抑制Keap1-Nrf2通路有关。而青光眼的发病机制较复杂，鼠尾草酸的生理活性多样，其可能通过调控不同通路影响青光眼的发生发展，其具体机制有待进一步开发，而本研究只是从动物层面进行初步探究，后续将增加实验设计，丰富细胞机制研究，为临床治疗提供理论依据。

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