·基础研究·

槲皮素对D-半乳糖致衰老小鼠模型学习记忆及脑内炎症的影响

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【摘 要】 **目的** 探讨槲皮素对D-半乳糖致衰老小鼠模型学习记忆能力及脑内炎症通路的影响。**方法** 将昆明小鼠32只随机分为对照组(NC组)、模型组(M组)、Q1治疗组(Q1组)、Q2治疗组(Q2组),每组8只。M组、Q1组及Q2组小鼠皮下注射D-半乳糖100 mg/(kg·d)建立小鼠衰老模型,NC组给予等量生理盐水。Q1、Q2组造模同时给予不同剂量槲皮素5、10 mg/(kg·d)灌胃干预,NC组和M组给予相同剂量生理盐水灌胃处理,干预8周。Morris水迷宫评价逃避潜伏期、穿越平台次数及平台象限滞留时间,Western blotting检测小鼠脑内高级糖基化终末产物(AGEs)及其受体(RAGE)、核转录因子(NF-κB)和其下游炎症因子肿瘤坏死因子-α(TNF-α)、白细胞介素-1β(IL-1β)、环氧化酶-2(COX-2)表达水平。采用SPSS 20.0统计软件对数据进行分析。组间比较采用完全随机设计的单因素方差分析或t检验。**结果** 与NC组比较,M组小鼠逃避潜伏期延长、穿越平台次数及在平台所在象限滞留时间降低,脑内AGEs、RAGE、NF-κB及炎症因子TNF-α、IL-1β、COX-2水平显著升高($P<0.05$)。与M组比较,治疗组小鼠逃避潜伏期缩短、穿越平台次数及在平台所在象限滞留时间延长,脑内炎性因子水平减少($P<0.05$)。与Q1组比较,Q2组小鼠逃避潜伏期缩短、穿越平台次数及在平台所在象限滞留时间延长,RAGE、IL-1β和COX-2表达水平显著降低。**结论** 槲皮素能够有效改善衰老小鼠学习记忆功能障碍,抑制AGEs/RAGE/NF-κB炎症通路,降低脑内炎性因子的生成,具有抗衰老潜力。

【关键词】 炎症;槲皮素;糖基化终末产物,高级;糖基化终末产物受体;D-半乳糖
【中图分类号】 R592;R364.5 【文献标志码】 A 【DOI】 10.11915/j.issn.1671-5403.2018.09.158

Effects of quercetin on learning and memory and cerebral inflammation in the D-galactose-induced aging mice

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【**Abstract**】 **Objective** To investigate the effects of quercetin on learning and memory and pathway of cerebral inflammation in D-galactose-induced aging mice. **Methods** Thirty-two Kunming mice were randomly divided into control group (NC group), model group (M group), Q1 treatment group (Q1 group) and Q2 treatment group (Q2 group), with 8 in each group. The aging mouse model was induced by subcutaneous administration of D-galactose 100 mg/(kg·d) in M, Q1 and Q2 groups, and the same amount of normal saline was administered in the same way in the NC group. Quercetin were then given in different doses in Q1 [5 mg/(kg·d)] and Q2 [10 mg/(kg·d)] groups, and the same dose of saline was given to the NC group and M group. Intervention duration was 8 weeks. Morris water maze was used to assess the escape latency, frequency of crossing the platform and retention time in the platform quadrant in the mice. Western blotting was employed to detect the expression of advanced glycation end products (AGEs) and their receptor (RAGE), nuclear factor-κB (NF-κB) and its downstream inflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and cyclooxygenase-2 (COX-2). SPSS statistics 20.0 was used for data analysis, and one-way ANOVA for complete randomized design or Student's t test for comparison between the groups. **Results** Compared with NC group, M group had lower escape latency, fewer crossings and shorter retention in target quadrant, but significantly higher AGEs, RAGE, NF-κB, TNF-α, IL-1β and COX-2. Compared with M group, treatment groups had lower escape latency, more crossings and longer retention in the quadrant of the platforms, and lower indices of cerebral inflammation ($P<0.05$). Compared with Q1 group, Q2 group had lower escape latency, more crossings and longer retention in the quadrant of platforms, and significantly lower expression of RAGE, IL-1β and COX-2. **Conclusion** Quercetin could effectively improve learning and memory dysfunction in the aging mice and reduce production of inflammatory factors in the brain by inhibiting AGEs/RAGE/NF-κB inflammatory pathway, hence having antiaging potential.

收稿日期:2018-05-21;**修回日期**:2018-06-14
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【Key words】 inflammation; quercetin; glycation end products, advanced; receptor for glycation end products; D-galactose
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衰老是中枢神经系统各种生理功能受损的主要因素,与进行性记忆力减退、认知障碍及痴呆等疾病密切相关。炎症在中枢神经系统的防御过程中发挥重要作用,与神经元缺失及包括阿尔茨海默病和帕金森病在内的多种神经退行性疾病相关[1]。研究表明,炎症可以增加淀粉样蛋白的聚集最终导致突触损伤,同时激活线粒体凋亡通路等[2,3]。D-半乳糖在体内的大量聚集一方面提高氧化应激水平,另一方面可以与蛋白质或多肽链生成高级糖基化终末产物(advanced glycation end products,AGEs)[4]。AGEs与其受体(receptor for advanced glycation end products,RAGE)结合参与衰老过程、炎症、神经退行性疾病的发生发展[5]。

黄酮类化合物主要由苯并吡喃和苯环组成,广泛存在于多种天然植物中,通过多种生理机制表现出神经保护功效,包括调节脑内免疫系统、抑制细胞因子释放等[6,7]。槲皮素是日常饮食中存在的主要黄酮类化合物之一,具有扩张冠状动脉、抗血小板聚集和很强的抗炎、抗氧化活性,可以预防神经系统退行性疾病、糖尿病、癌症、肥胖症等多种疾病[8]。然而在退行性疾病发生之前的衰老阶段,槲皮素能否延缓脑老化的研究较少。已有研究发现,槲皮素通过降低脑内氧化应激水平、调节突触内钙稳态、增加Na^+-K^+-ATP酶活性、增加生长相关蛋白43(growth associated protein 43, GAP43) mRNA的表达,减轻D-半乳糖所致的神经毒性,改善小鼠行为学损伤[9]。但对AGEs/RAGE炎症通路的作用研究较少。本研究旨在探讨槲皮素对D-半乳糖所致衰老小鼠学习记忆能力以及脑内AGEs/RAGE通路水平的影响。

1 材料与方法

1.1 材料

6周龄雄性昆明小鼠32只,体质量(30±4)g(山东大学动物中心);槲皮素、D-半乳糖(sigma公司);兔抗AGEs、肿瘤坏死因子-α(tumor necrosis factor-α,TNF-α)、白细胞介素-1β(interleukin-1β,IL-1β)及环加氧酶-2(cyclooxygenase-2,COX-2)多克隆抗体(Abcam公司),兔抗RAGE多克隆抗体及小鼠抗β-actin多克隆抗体(Santa Cruz公司),兔抗核转录因子κB(nuclear factor κB,NF-κB)多克隆抗体(Millipore公司),蛋白印迹相关二抗(中杉金桥),组织总蛋白抽提试剂盒、BCA蛋白浓度测定试剂盒(碧云天生物技术有限公司);ECL发光液(Millipore公司)。

1.2 方法

1.2.1 动物分组 随机将小鼠分为对照组(NC组)、模型组(M组)、Q1治疗组(Q1组)和Q2治疗组(Q2组),每组8只。M组、Q1组、Q2组小鼠行颈背部皮下注射D-半乳糖100 mg/(kg·d),NC组给予等量生理盐水皮下注射。Q1、Q2组造模同时分别给予槲皮素5、10 mg/(kg·d)灌胃干预[10],NC组和M组给予相同剂量生理盐水灌胃处理,干预8周,及时补充实验过程中意外死亡小鼠。

1.2.2 Morris水迷宫试验 水迷宫设备是直径100 cm、高60 cm的圆形水池,以2条垂直的直径为界限将水池平分为4个象限,高约40 cm的平台置于第一象限,水位高出平台1 cm。定位航行实验和空间探索实验评估小鼠空间学习、记忆能力。(1)定位航行实验:每天训练4次,训练5 d,将小鼠按照象限顺序面向池壁沿池壁各象限边缘中点放入水中,记录小鼠入水至找到平台并停留10 s所用时间,即逃避潜伏期(escape latency,EL)。若停留不足10 s,则重新入水继续记录时间;若小鼠60 s内未能成功找到平台,则协助其至平台并停留15 s,此时EL记为60 s。(2)空间探索实验:第6天撤除平台行空间探索实验,于第三象限将小鼠沿池壁放入,记录小鼠60 s内第1次到达原平台所在区域形成的EL、穿越平台所在象限的次数及在平台所在象限滞留时间。

1.2.3 脑组织取材及总蛋白提取 腹腔注射3%苯巴比妥钠将小鼠麻醉,断头取脑。称取脑组织100 mg,加入裂解液500 μl、蛋白酶抑制剂5 μl,制备组织匀浆,静置30 min,4℃离心30 min,吸取上清液,-80℃保存,用于Western blotting蛋白检测。

1.2.4 蛋白Western blotting分析 BCA法测蛋白浓度,蛋白变性后经聚丙烯酰胺琼脂糖凝胶电泳,随后转移至聚偏二氟乙烯膜(polyvinylidene fluoride, PVDF)上,加入AGEs、RAGE、NF-κB、TNF-α、IL-1β及COX-2一抗(1∶1000)4℃过夜;二抗室温孵育1 h,加入ECL发光液显影。

1.3 统计学处理

采用SPSS 20.0统计软件对数据进行分析。计量资料以均数±标准差($\bar{x}\pm s$)表示,组间比较采用t检验和方差分析。$P<0.05$为差异有统计学意义。

2 结 果

2.1 3组小鼠空间学习和记忆能力比较

与NC组比较,M组小鼠到达平台的EL明显延长,穿越平台次数明显减少,平台所在象限滞留时间显著降低($P<0.05$),提示D-半乳糖皮下注射可以导致小鼠空间学习和记忆能力损害。与M组比较,Q1组小鼠在平台所在象限滞留时间明显延长($P<0.05$),Q2组小鼠EL明显缩短,穿越平台次数显著增加,小鼠在平台所在象限滞留时间增加($P<0.05$)。与Q1组比较,Q2组EL缩短、穿越平台次数增加、小鼠在平台所在象限滞留时间增加,差异均有统计学意义($P<0.05$;图1)。

2.2 3组小鼠脑组织AGEs、RAGE及NF-κB表达水平比较

与NC组相比,M组小鼠AGEs、RAGE及NF-κB表达水平显著增高($P<0.05$);与M组相比,Q1组小鼠AGEs表达水平显著降低($P<0.05$),Q2组小鼠AGEs、RAGE和NF-κB表达显著降低($P<0.05$);与Q1组比较,Q2组小鼠RAGE表达显著下降($P<0.05$),其他差异无统计学意义($P>0.05$;图2)。

2.3 3组小鼠脑组织TNF-α、IL-1β及COX-2表达水平比较

与NC组相比,M组小鼠脑组织TNF-α、IL-1β和COX-2表达水平显著增加($P<0.05$),提示M组脑内炎性反应显著;与M组比较,Q1组和Q2组TNF-α、IL-1β和COX-2表达水平明显降低($P<0.05$);与Q1组比较,Q2组小鼠IL-1β和COX-2表达水平显著降低,TNF-α水平在2组间差异无统计学意义($P>0.05$;图3)。

3 讨 论

既往研究发现,槲皮素对于过氧化氢、脂质过氧化物、6-羟多巴胺、β淀粉样肽25~35及脂多糖等多种物质所致的神经毒性具有较好的保护作用[11],同时也可减轻帕金森病、痴呆、高胆固醇所致神经病变模型动物的脑损害[12-15]。本研究探讨了槲皮素对D-半乳糖所致的衰老小鼠学习和记忆能力障碍、炎症通路AGEs/RAGE/NF-κB的作用。结果发现,长期D-半乳糖颈背部皮下注射可以导致小鼠明显的空间学习和记忆能力障碍,增加脑内AGEs及其受体RAGE的表达,诱导NF-κB活化及下游炎性因子表达。采用槲皮素治疗能够逆转D-半乳糖所致的学习和记忆障碍,下调AGEs/RAGE/NF-κB通路中的炎性因子表达。

D-半乳糖是一种神经毒物质,可以很容易与蛋白质和肽中氨基酸的游离胺反应,从而诱导AGEs生成[16]。众所周知,AGEs是一种有害的翻译后蛋白修饰,可以诱导过量活性氧(reactive oxygen species,ROS)生成及异常氧化应激水平增高,加速衰老过程[17]。此外,越来越多的证据表明,AGEs与细胞表面受体如RAGE结合,介导NF-κB活化,从而最终导致氧化应激、炎症反应、神经变性及记忆力受损[5]。因此针对AGEs/RAGE/NF-κB介导的炎症反应的靶向药物对于研究多种年龄相关疾病的进展及治疗方面具有很好的治疗潜力。

已有研究表明,槲皮素具有很强大的抗炎能力,在多种自身免疫性小鼠模型中发挥炎症和免疫调节活性。如槲皮素可改善角叉菜胶和高脂饮食诱导的炎症反应,降低佐剂诱导的大鼠慢性关节炎[18];在肥胖的Zucker大鼠中,槲皮素可减少内脏脂肪组织

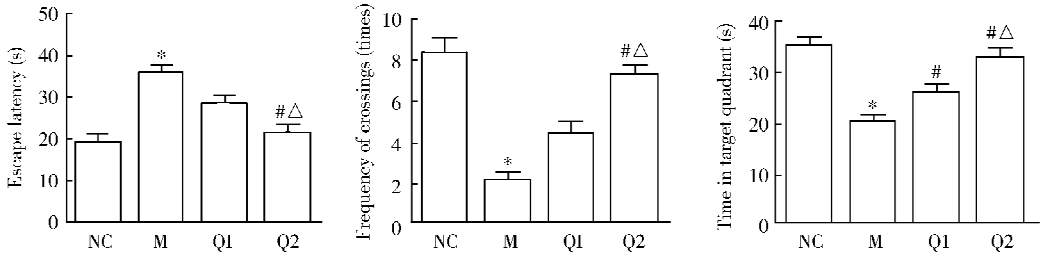


图1 Morris水迷宫试验逃避潜伏期、穿越平台次数及平台象限滞留时间

Figure 1 The result of escape latency, frequency of crossing the platform and platform quadrant retention time from Morris water maze test

NC: control group; M: model group; Q1: 5 mg/(kg·d) group; Q2: 10 mg/(kg·d) group. Compared with NC group, $^{*}P<0.05$; compared with M group, $^{\#}P<0.05$; compared with Q1 group, $^{\triangle}P<0.05$

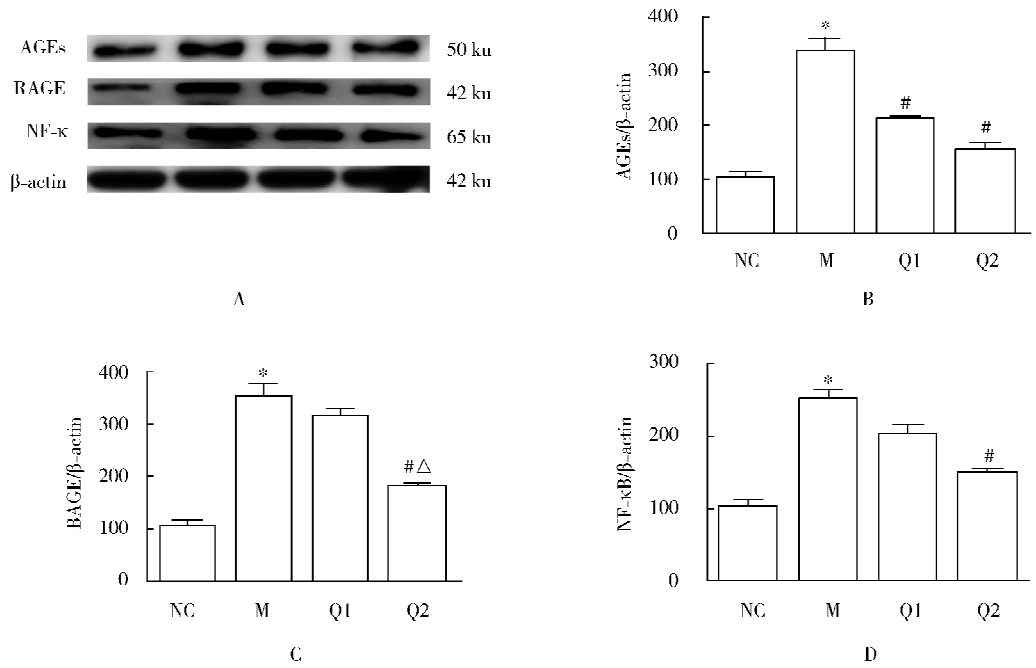


图2 Western blotting检测小鼠脑内AGEs,RAGE,NF-κB蛋白表达

Figure 2 Expression of AGEs, RAGE and NF-κB in the brain of mice by Western blotting

AGEs: advanced glycation end products; RAGE: receptor for advanced glycation end products; NF-κB: nuclear factor-κB. A: Western blotting; B, C, D: statistical analysis of protein. NC: control group; M: model group; Q1: 5 mg/(kg·d) group; Q2: 10 mg/(kg·d) group. Compared with NC group, $^{*}P<0.05$; compared with M group, $^{\#}P<0.05$; compared with Q1 group, $^{\triangle}P<0.05$

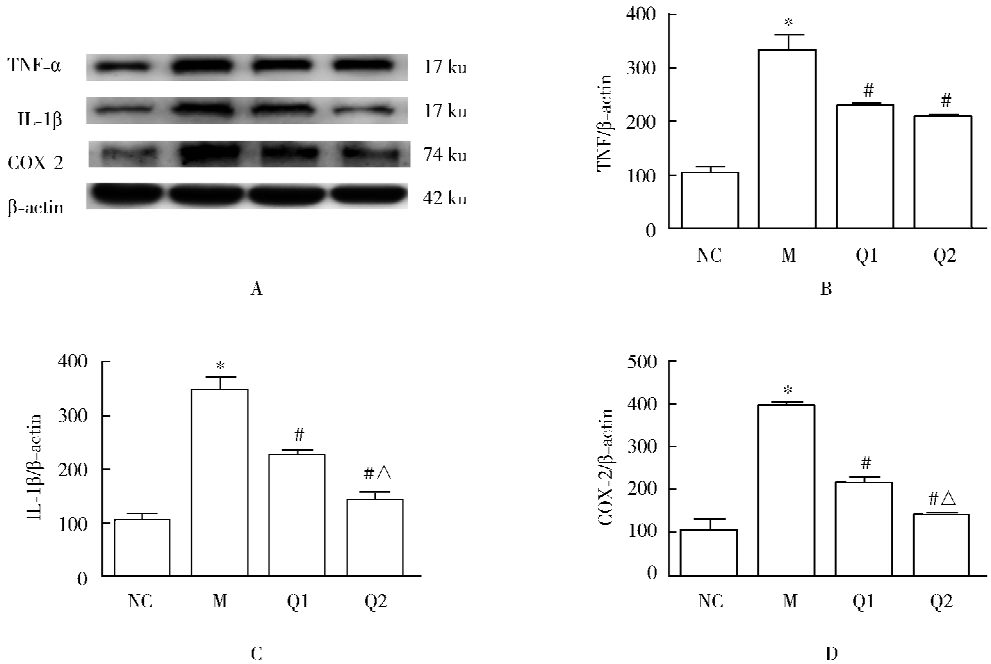


图3 Western blotting检测小鼠脑内TNF-α、IL-1β、COX-2表达

Figure 3 Expression of TNF-α, IL-1β and COX-2 in the brain of mice by Western blotting

TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; COX-2: cyclooxygenase-2. A: Western blotting; B, C, D: statistical analysis of protein. NC: control group; M: model group; Q1: 5 mg/(kg·d) group; Q2: 10 mg/(kg·d) group. Compared with NC group, $^{*}P<0.05$; compared with M group, $^{\#}P<0.05$; compared with Q1 group, $^{\triangle}P<0.05$

TNF-α和一氧化氮的产生,并下调一氧化氮合酶的表达[19],提示槲皮素对于多种炎症模型动物具有较强的抗炎作用。对其抗炎机制的深入探讨发现,一方面槲皮素可以通过螯合金属离子、捕获甲基乙二

醛、捕获 ROS，从而抑制 AGEs 形成[20]；另一方面，槲皮素可通过直接捕获甲基乙二醛和乙二醛，显著抑制 AGEs 的形成[21]。而我们对衰老模型小鼠的研究进一步验证了槲皮素对 AGEs 的抑制作用，并发现其对 AGEs 相关通路 RAGE/NF-κB 及下游炎症因子表达水平的明显抑制作用。

细胞因子和其他炎性介质的产生可导致神经元凋亡，进而加重退行性病变[1]。既往研究表明，长期 D－半乳糖皮下注射可增加 ROS 及脑内氧化应激水平，模型动物出现突触损伤、胶质细胞激活、脑内炎症反应，最终导致脑老化[17]。本研究采用 Morris水迷宫试验检测到长期 D－半乳糖干预导致了小鼠记忆力受损程度，但槲皮素治疗逆转了 D－半乳糖所致的学习和记忆力障碍，表现为小鼠 EL 明显缩短、穿越平台次数显著增加、平台象限滞留时间延长，与模型组比较，剂量组炎性因子水平呈浓度依赖性减低，推测槲皮素可能通过减轻炎症因子对脑组织的损伤而改善认知功能障碍。

综上所述，本研究通过长期皮下注射 D－半乳糖建立衰老小鼠模型，并采用槲皮素给予灌胃干预，发现槲皮素可显著改善衰老小鼠学习和记忆能力，降低脑内炎症反应，下调 AGEs/RAGE/NF-κB 通路中炎性因子的表达。针对槲皮素对脑老化的保护作用及其机制的深入研究，可为其应用于延缓脑衰老，预防阿尔茨海默病、帕金森病等神经系统退行性病变提供理论依据。

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（编辑：张芙）