·基础研究·

葡萄糖限制对丙烯醛诱导的永生化小鼠海马神经元神经毒性的保护作用

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摘 要：【目的】探讨葡萄糖限制(GR)对丙烯醛诱导的神经毒性及AD病理的影响。【方法】永生化小鼠海马神经元HT22细胞预先用不同浓度葡萄糖(25 mmol/L、17.5 mmol/L、12.5 mmol/L)的培养基处理，随后用丙烯醛(25 μmol/L)孵育指定时间后，采用MTT法和流式细胞术分别检测细胞活力和凋亡情况，并通过酶联免疫吸附试验(ELISA)试剂盒检测谷胱甘肽(GSH)、超氧化物歧化酶(SOD)和丙二醛(MDA)的变化。Western blot检测神经营养因子BDNF/TrkB信号蛋白及参与淀粉样前体蛋白(APP)代谢关键酶的表达。【结果】研究结果显示，GR显著改善丙烯醛暴露诱导的HT22细胞活力下降，抑制丙烯醛暴露引起的GSH和SOD耗竭，并降低了MDA水平，改善了丙烯醛诱导的氧化应激损伤($P<0.05$)。此外，GR可抑制丙烯醛诱导的早期细胞凋亡，并上调BDNF/TrkB信号，保护性调节APP代谢相关酶(ADAM-10与BACE1)的表达($P<0.05$)。【结论】GR对丙烯醛诱导的神经毒性具有保护作用，其在预防或延缓阿尔茨海默病发展中具有潜在作用。

关键词：葡萄糖限制；丙烯醛；阿尔茨海默病；Aβ沉积；氧化应激损伤，*BDNF/TrkB*信号通路
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Protective Effects of Glucose Restriction Against Acrolein-induced Neurotoxicity in Hippocampal Cells of Immortalized Mice

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Abstract:【Objective】to evaluate the effects and underlying mechanisms of a glucose restriction (GR) regimen on acrolein-induced neurotoxicity and AD-related pathology.【Methods】Immortalized mouse hippocampal HT22 cells were pretreated with culture media containing different glucose concentrations (25 mmol/L, 17.5 mmol/L, and 12.5 mmol/L), followed by incubation with 25 μmol/L acrolein for specified durations. Cell viability and apoptosis were assessed using MTT assay and flow cytometry, respectively. Changes in glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were measured using enzyme linked immunosorbent assay(ELISA) kits. Alterations in the expression of BDNF/TrkB signaling proteins and key enzymes involved in amyloid precursor protein (APP) metabolism were evaluated by Western blot.【Results】Pretreatment with GR significantly attenuated acrolein-induced loss of cell viability in HT22 cells. GR also alleviated the depletion of GSH and SOD, reduced MDA levels, and thereby ameliorated

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acrolein-induced oxidative damage($P<0.05$). Furthermore, GR inhibited acrolein-triggered early apoptosis. Notably, the GR regimen protectively modulated the expression of BDNF/TrkB signaling pathway components and key APP-metabolizing enzymes (ADAM10 and BACE1)($P<0.05$).【Conclusion】GR exerts neuroprotective effects against acrolein-induced toxicity, suggesting its potential in preventing or delaying the development of AD.

Key words: glucose restriction; acrolein; Alzheimer's disease; Aβ deposition; oxidative damage; BDNF/TrKB

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丙烯醛是一种高活性的α,β-不饱和醛,广泛存在于油炸食品(如甜甜圈)、咖啡、烟草烟雾及汽车尾气中[1]。临床研究表明,神经退行性疾病患者脑脊液中丙烯醛水平显著升高[2-3],其通过诱导氧化应激加剧神经元损伤的机制已被证实与阿尔茨海默病(Alzheimer's disease, AD)病理密切相关[2,4-5]。因此,靶向丙烯醛毒性通路可能成为神经退行性疾病治疗的新方向。近年来,饮食因素在神经系统相关疾病中的作用受到越来越多关注。适度的热量限制(calorie restriction,CR)是指在不造成营养不良的情况下适当减少能量摄入[6]。研究已经明确证实,热量限制可以在从酵母到非灵人长类动物的各种动物模型中减缓衰老并增加最大寿命[7-8]。有趣的是,流行病学数据显示,低热量饮食可以降低AD和帕金森病的风险[9-11]。蔗糖是饮食中最常摄入的糖类,它由等量的葡萄糖和果糖组成,因此葡萄糖是最主要的日常能量来源[12]。此外,在大多数真核细胞中,葡萄糖亦是主要的能量来源。葡萄糖限制(glucose restriction,GR)对代谢、应激反应、细胞活性调节和凋亡的潜在机制研究已经广泛开展[13-14]。然而,关于GR是否对神经退行性疾病具有保护作用仍然存在争议。CR具有抗氧化和抗凋亡作用,并能保护性调节神经营养因子如脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)、神经营养因子-3(neurotrophin-3, NT-3)和胶质细胞源性神经营养因子(glial cell line-derived neurotrophic factor, GDNF)的表达,进而产生神经保护效应[9,15-16]。然而,GR的神经保护作用效应及机制则尚未完全明确。在本研究中,我们探讨了GR对丙烯醛诱导的神经毒性和AD样病理的影响及其潜在机制。

1 材料与方法

1.1 主要试剂与仪器

DMEM高糖培养基(货号11965175)、DMEM无糖培养基(货号A1443001)、胎牛血清(fetal bovine serum,FBS,货号30044333)、2.5 g/L胰蛋白酶(货号15050057)均购自Gibco。丙烯醛购自成都格雷西亚化学试剂公司。蛋白免疫印迹实验中所用异丙醇、无水乙醇等常用化学试剂均购自广州化学试剂厂,所用RIPA裂解液、蛋白酶抑制剂、磷酸酶抑制剂、蛋白上样缓冲液、BCA蛋白浓度检测试剂盒均购自碧云天。除非另有说明,D-葡萄糖、3-(3,4-二巯基噻唑-2-基)-2,5-二苯基溴化四氮唑(MTT)和所有其他化学试剂均购自Sigma-Aldrich。

1.2 细胞培养和细胞处理

永生化小鼠HT22海马细胞由中山大学孙逸仙纪念医院刘军教授实验室提供[17]。葡萄糖是中枢神经系统所需的基本能量物质[18]。HT22细胞作为一种永生化的小鼠海马神经元,由于代谢需求高,在正常生长培养基中需要25 mmol/L葡萄糖[19]。一般来说,实验研究中常用的热量限制(CR)方案为20%~40%,而严重的CR或GR超过50%则会产生有害影响,导致营养不良[20-22]。因此,本研究选择葡萄糖浓度分别为17.5 mmol/L和12.5 mmol/L,分别代表30%和50%的GR方案进行实验。

首先,将HT22细胞置于不同葡萄糖浓度(25 mmol/L、17.5 mmol/L和12.5 mmol/L)的培养基中培养6~48 h,探索最佳的GR干预方案。随后,选择葡萄糖浓度和处理时间最合适的培养基作为最终GR方案应用于后续实验,同时以含25 mmol/L葡萄糖的普通培养基作为对照。收集对数生长期细胞以每孔4 000个的密度接种于96孔板,采用GR培养基或普通培养基预处理指定时间后,加入25 μmol/L的丙烯醛再继续培养6~24 h。

1.3　细胞活力检测

采用MTT试剂盒检测细胞活力，具体操作步骤按照说明书并参照本课题组前期研究[23]中描述的方法进行。简言之，将细胞按4 000个/孔的密度接种到96孔板中，接收GR及丙烯醛暴露处理后，移去培养基，加入10 μL MTT溶液（5 mg/mL原液）后37 ℃下继续孵育1 h。孵育结束后，移除MTT溶液并加入150 μL的二甲基亚砜终止反应。采用酶标仪（Bio-Tek）检测570 nm波长处的吸光度。同时设置调零孔和空白孔（培养基及MTT）。细胞存活率=干预组吸光值/空白对照组吸光值×100%。空白对照组细胞存活率设为100%。

1.4　细胞内谷胱甘肽的测定

细胞内谷胱甘肽（glutathione，GSH）浓度用GSH测定试剂盒（南京建成生化）测定，具体操作方法如本课题前期研究中所述[23]。细胞经过相应处理后采用4 ℃裂解缓冲液裂解细胞，在4 ℃下以10 000×*g*离心15 min后收集上清液，在1 mL上清液中加入50 μL TEAM试剂。在每个样品孔中加入50 μL样品或试剂盒中提供的标准溶液。将150 μL新鲜制备的检测混合物加入到每个含有标准品和样品的孔中。将孔板放在轨道摇床上室温暗处孵育25 min后，使用酶标仪（Bio-Tek）检测405 nm波长处吸光度，绘制标准曲线，计算各样品的GSH浓度（样品GSH最终浓度用所测出浓度除以样品蛋白浓度以校正结果）。

1.5　超氧化物歧化酶活性和丙二醛水平检测

超氧化物歧化酶（superoxide dismutase，SOD）活性和丙二醛（malondialdehyde，MDA）水平用商用检测试剂盒（中国建诚生化）进行检测，具体操作方法如本课题前期研究中所述[23]。细胞经过相应处理后采用4 ℃裂解缓冲液裂解细胞，在4 ℃下以10 000×*g*离心15 min。收集上清液，以还原型烟酰胺腺嘌呤二核苷酸为底物，评估SOD活性（以单位/毫克蛋白质表示，U/mg），一个单位指能够抑制显色反应50%所需的量。MDA（以毫摩尔/毫克蛋白质表示，nmol/mg）反映脂质过氧化水平，采用MDA检测试剂盒按照标准方案检测硫代巴比妥酸活性物质来测定。同时用BCA检测试剂盒（Beyotime，中国江苏）测定蛋白质含量。

1.6　细胞凋亡的流式细胞分析

流式细胞仪分析参考本课题组前期的研究方法[23]进行。一般情况下，处理后的细胞经胰蛋白酶消化后收集，然后用磷酸盐缓冲盐水（PBS，pH 7.4）洗涤一次。离心后，用annexin V和碘化丙啶（PI）对细胞进行染色，以区分早期凋亡（右下象限，annexin V+/PI-细胞）、晚期凋亡（右上象限，annexin V+/PI+细胞）和坏死（左上象限，annexin V-/PI+细胞）细胞群；左下象限（annexin V-/PI-细胞）为活细胞，用annexin V-FITC细胞凋亡检测试剂盒（BD Biosciences，San Jose，CA）进行检测。annexin V和PI双染色过程严格按照生产商的说明进行。同时检测caspase-3/7或Bcl-2的水平，采用caspase-3/7工作反应试剂盒（Millipore，MA，USA）或Bcl-2工作反应试剂盒（Millipore，MA，USA）在室温下暗处孵育细胞10 min，使用Muse细胞分析仪（Millipore，MA，USA）测定凋亡细胞、caspase-3阳性细胞和活化的Bcl-2阳性细胞的数量。

1.7　蛋白免疫印迹

参照本课题组已发表的研究[23]中所述的方法进行蛋白免疫印迹实验。简言之，细胞用冰PBS冲洗两次，然后加入100 μL裂解缓冲液（20 mmol/L Tris-HCl、150 mmol/L NaCl、1 mmol/L EDTA、10 g/L Triton X-100、蛋白酶抑制剂鸡尾酒、2 mmol/L Na_3VO_4和10 mmol/L NaF，pH 7.5）充分裂解。用BCA检测试剂盒（碧云天，中国江苏）测定蛋白质的浓度，然后以相同的量（20 μg）载入各泳道。蛋白质经SDS-PAGE凝胶电泳分离后，电转移到PVDF膜（Millipore）上，采用脱脂牛奶（50 g/L）室温封闭1-2 h，封闭结束后依次加入稀释好的一抗（Anti-*BDNF*，1∶1 000，Millipore，美国；Anti-*TrKB*，1∶500，Abcam，美国；Anti-*APP*，1∶2 000，Gene Tex，美国；Anti-*BACE1*，1∶1 000，Millipore，美国；Anti-*ADAM10*，1∶1 000，Millipore，美国；Anti-β-*actin*，CST，美国，1∶1 000）、二抗【Anti-Rabbit IgG（HP）】，1∶2 000，CST，美国；Anti-Mouse（HP），1∶10 000，Promega，美国）孵育，孵育结束后使用ECL plus试剂盒（Pierce）进行自动放射显像。使用Quantity One软件（Bio-Rad，Hercules，CA）进行定量分析。

1.8　统计分析方法

采用SPSS 20.0统计软件包进行统计学处理，本研究中描述的所有定量数据和实验均重复至少3次。数据以多个独立实验的平均值±标准差表示，多组间比较，采用单因素方差分析并用

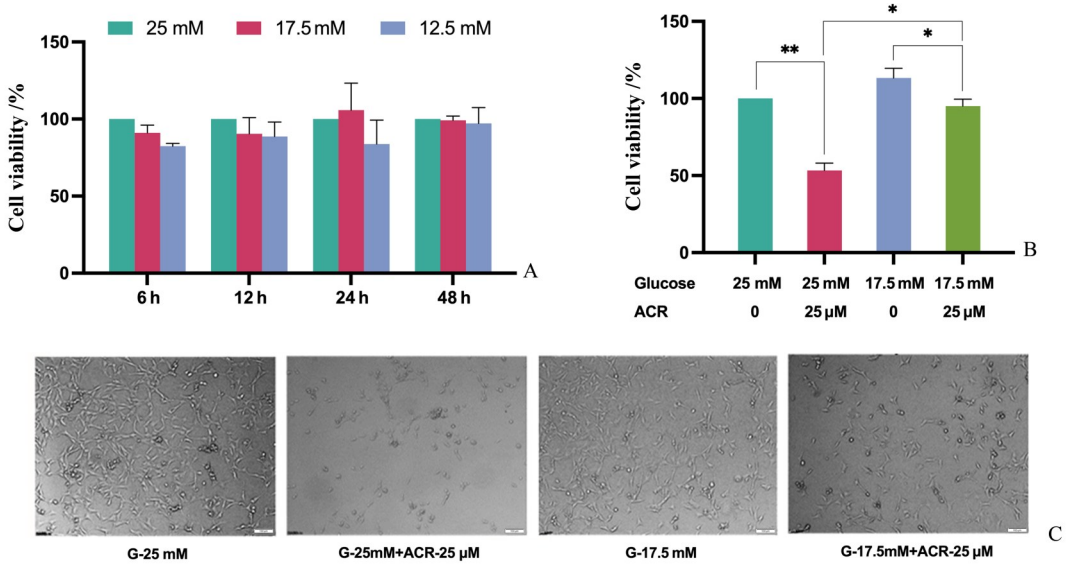
Dunnett's 法进行多重比较(数据满足方差齐性时)或非参数秩和检验(Kruskal-Wallis *H* 检验)并用 Dunnett's 进行多重比较(数据不满足方差齐性时),以确定统计差异。$P<0.05$(双侧)被认为具有统计学差异。

2 结 果

2.1 葡萄糖限制对永生化小鼠海马神经元细胞的保护作用

为了验证葡萄糖限制(GR)是否能够保护永生化小鼠海马神经元(HT22)细胞免受丙烯醛诱导的神经毒性影响,本研究首先探讨了GR对丙烯醛诱导的细胞死亡和形态变化的作用。实验结果显示,当葡萄糖浓度为17.5 mmol/L时,细胞存活率在24 h内达到最佳;而当葡萄糖浓度限制在12.5 mmol/L时,细胞存活率略有下降(图1A)。

随后,用不同浓度的葡萄糖(17.5 mmol/L或20 mmol/L)培养液预处理HT22细胞24 h,再用丙烯醛(25 μmol/L)处理24 h。结果显示,在17.5 mmol/L葡萄糖组中,经丙烯醛处理后的细胞活力仅下降至(89±12)%,细胞形态的破坏程度也明显低于25 mmol/L葡萄糖组(图1B,C)。



A: Effects of two different GR regimen on HT22 cells viability at indicated time points (6-48 h).**B:** Effects of 17.5 mmol/L of glucose on acrolein-induced viability loss. $n=3$, $F=96.12$, $P<0.000\ 1$. $^{**}P<0.01$, $^{*}P<0.05$ versus indicated groups. **C:** Phase contrast microphotographs (100×) of HT22 cells pretreated with different concentration of glucose for 24 h then incubation with 25 μmol/L acrolein for another 24 h. G: glucose; ACR: acrolein; mM: mmol/L; μM: μmol/L.

图1 葡萄糖限制对HT22细胞中丙烯醛诱导的神经毒性的影响

Fig. 1 Effects of GR on acrolein-induced neurotoxicity in HT22 cells

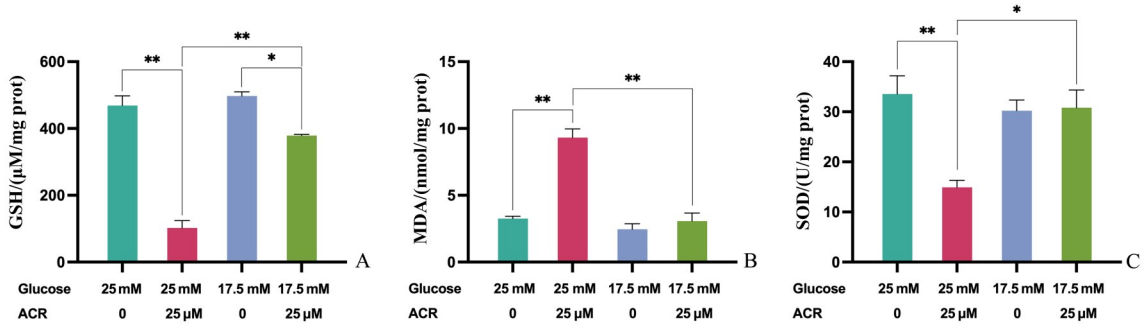
2.2 葡萄糖限制对丙烯醛诱导的氧化损伤的缓解作用

本研究进一步探讨了葡萄糖限制(GR)在抑制丙烯醛诱导的氧化损伤中的潜在作用。通过检测细胞氧化损伤的标志性抗氧化剂(包括GSH和SOD)以及MDA的浓度,我们发现,在含25 mmol/L葡萄糖的培养基中,HT22细胞暴露于丙烯醛24 h后,MDA水平显著升高,而GSH和SOD活性显著降低。然而,当葡萄糖浓度限制在17.5 mmol/L时,对丙烯醛诱导的氧化损伤具有显著的保护作用,上述指标均显著恢复(图2)。

2.3 葡萄糖限制对丙烯醛诱导的永生化小鼠海马神经元细胞凋亡的抑制作用

本研究通过流式细胞分析评估了永生化小鼠海马神经元(HT22)细胞的凋亡情况。结果显示,用正常培养基(25 mmol/L葡萄糖)预处理的细胞暴露于丙烯醛6 h后,早期凋亡细胞数量显著增加(60.30±2.97)%。而用葡萄限制培养基(17.5 mmol/L葡萄糖)预处理后再暴露于丙烯醛时,早期凋亡细胞的数量明显减少(19.93±4.03)%(图3A,B)。

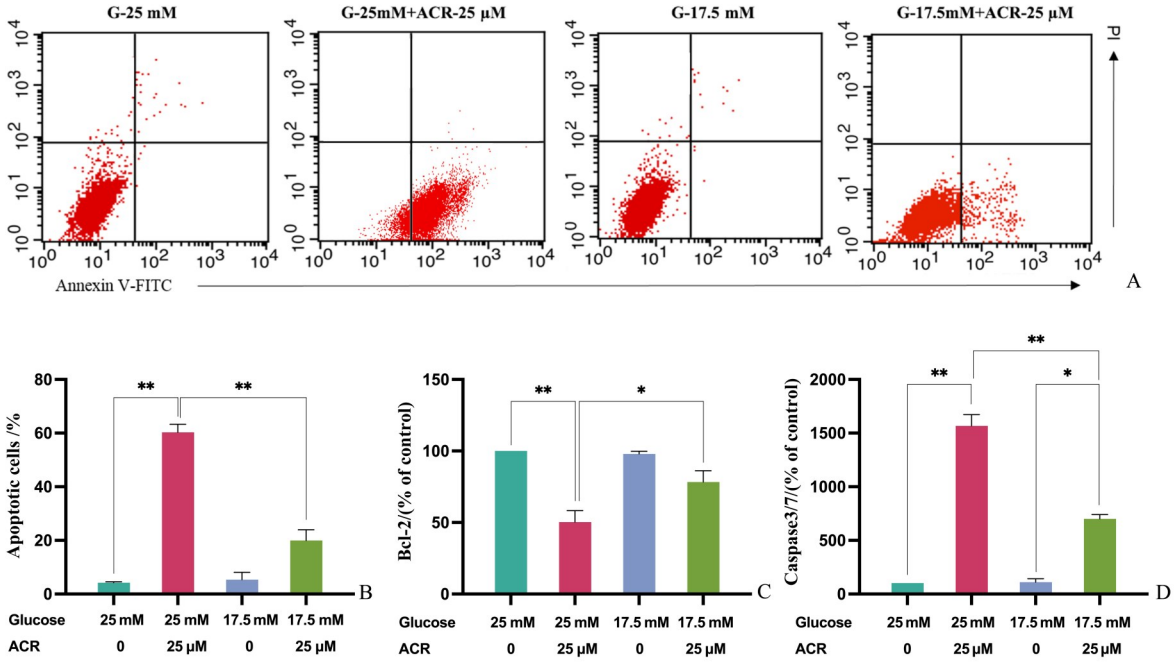
Bcl-2蛋白和Caspase 3/7的水平是调控细胞凋



Cells were pretreated for 24 hours with culture medium containing either 25 mmol/L or 17.5 mmol/L glucose, followed by incubation with 25 μmol/L acrolein for an additional 24 hours. Intracellular levels of GSH, MDA, and SOD were subsequently measured. A: GSH. n=3, F=261.8, P<0.000 1. $^{**}P$<0.01, $^{*}P$<0.05 versus indicated groups. B: MDA. n=3, F=124.3, $^{**}P$<0.01 versus indicated groups. C: SOD. n=3, F=26.53, P<0.001. $^{**}P$<0.01, $^{*}P$<0.05 versus indicated groups. SOD: superoxide dismutase; MDA: malondialdehyde; GSH: glutathione; ACR: acrolein; mM: mmol/L; μM: μmol/L.

图2　葡萄糖限制对HT22细胞中丙烯醛诱导的氧化损伤的影响

Fig. 2　Effects of GR on acrolein-induced oxidative damage in HT22 cells



HT22 cells were pretreated with culture medium containing different levels of glucose for 24 h before being treated with 25 μmol/L acrolein for another 6 h. The apoptotic cells were analyzed using an Annexin V/PI staining assay. The Bcl-2 level and the Caspase-3 level were measured by flow cytometry. A: Apoptosis detection with Annexin V/PI staining. B: Quantification of apoptotic cells by Annexin V-FITC/PI flow cytometry. n=3, F=254.1, P<0.000 1. $^{**}P$<0.01 versus indicated groups. C: Bcl-2. n=3, F=48.67, P<0.0001. $^{**}P$<0.01, $^{*}P$<0.05 versus indicated groups. D: caspase3/7. n=3, F=425.6, P<0.000 1. $^{**}P$<0.01, $^{*}P$<0.05 versus indicated groups. ACR: acrolein; mM: mmol/L; μM: μmol/L.

图3　葡萄糖限制对HT22细胞中丙烯醛诱导的细胞凋亡的影响

Fig. 3　Effects of GR on acrolein-induced apoptosis in HT22 cells

亡的关键因素。研究发现，用正常培养基（25 mmol/L葡萄糖）预处理的细胞暴露于丙烯醛后，Bcl-2蛋白表达明显降低，且活化的Caspase3/7蛋白表达明显升高（图3C，D）。而当培养基中葡萄糖水平限制至17.5 mmol/L水平时，Bcl-2的表达明显增加（图3C），活化Caspase3/7的水平则明显降低（图3D）。

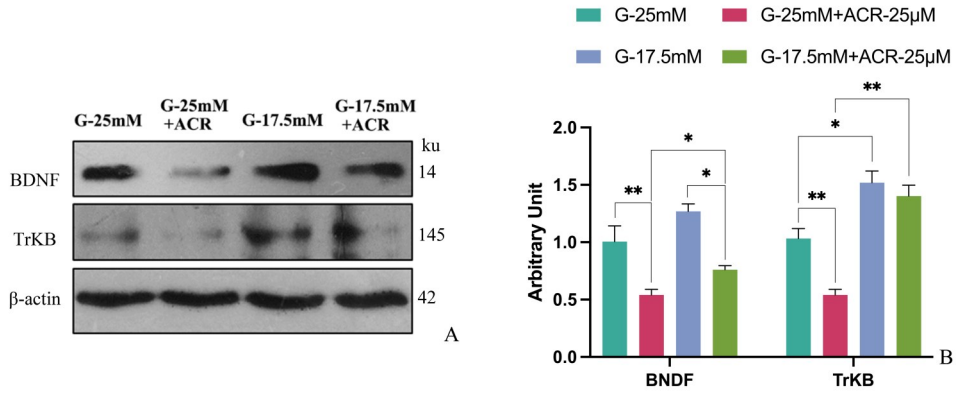
2.4　葡萄糖限制对丙烯醛诱导的BDNF/TrkB通路的改善作用

本研究还发现，单纯采用GR方案（17.5 mmol/L葡萄糖）处理HT22细胞，可上调BDNF及其受体酪

氨酸激酶B(tyrosine kinase B, TrkB)的表达。然而,用正常培养基(25 mmol/L葡萄糖)培养的HT22细胞,在暴露于丙烯醛之后,BDNF/TrkB信号通路会被显著抑制。有趣的是,若用葡萄糖限制培养基(17.5 mmol/L葡萄糖)对细胞进行预处理,在暴露于丙烯醛之后,BDNF/TrkB信号表达仅轻度下调(图4A,B)。

2.5 葡萄糖限制对阿尔茨海默病病理相关蛋白表达的调节作用

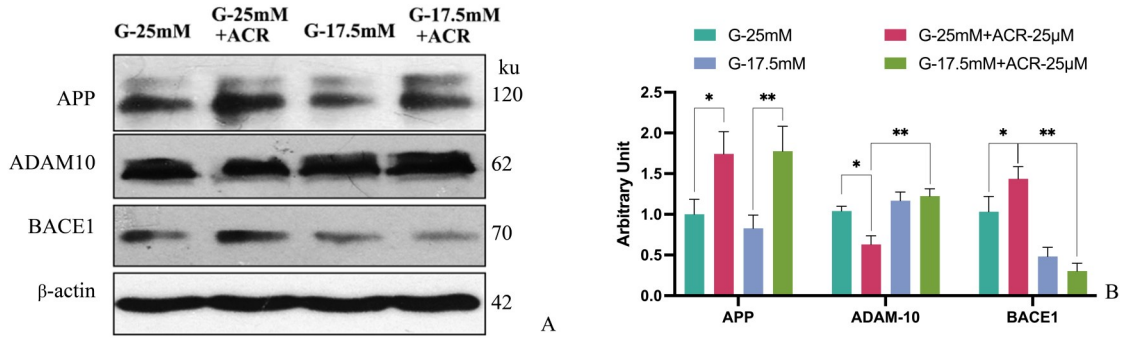
本研究进一步探讨了GR对AD病理相关蛋白表达的影响。结果表明,在正常培养基(25 mmol/L葡萄糖)培养条件下,HT22细胞暴露于丙烯醛后,淀粉样前体蛋白(amyloid precursor protein, APP)和β-分泌酶(beta-site amyloid precursor protein cleaving enzyme 1, BACE-1)的表达水平显著上调,而α-分泌酶相关蛋白解整合素-金属蛋白酶10(a disintegrin and metalloproteinase domain-containing protein 10, ADAM-10)的表达水平则显著下调。然而,当培养基中葡萄糖浓度限制为17.5 mmol/L(模拟GR状态)时,ADAM-10水平显著恢复,BACE-1水平显著降低,但APP水平未见明显变化(图5)。



A: HT22 cells were pretreated with culture medium containing different levels of glucose for 24 h before being treated with 25 μmol/L acrolein for another 24 h. The protein levels of BDNF and TrkB were measured by Western blotting. B: Densitometric measurements of band intensity were performed using Quantity One Software. *BDNF*: n=3, F=44.08, P<0.000 1. $^{**}P$<0.01, $^{*}P$<0.05 versus indicated groups. *TrKB*: n=3, F=79.28, P<0.000 1. $^{**}P$<0.01, $^{*}P$<0.05 versus indicated groups. ACR: acrolein; mM: mmol/L; μM: μmol/L.

图4 葡萄糖限制对HT22细胞中丙烯醛诱导的BDNF/TrkB通路抑制的影响

Fig. 4 Effects of GR on acrolein-induced inhibition of BDNF/TrkB pathway in HT22 cells



A: HT22 cells were pretreated with culture medium containing different levels of glucose for 24 h before being treated with 25 μmol/L acrolein for another 24 h. The protein levels of proteins were measured by Western blotting. B: Densitometric measurements of band intensity were performed using Quantity One Software. APP: n=3, F=12.79, P<0.01. $^{**}P$=0.002, $^{*}P$<0.05 versus indicated groups. ADAM-10: n=3, F=25.10, P=0.002. $^{**}P$<0.01, $^{*}P$<0.05 versus indicated groups. BACE1: n=3, F=41.24, P<0.001. $^{**}P$<0.01, $^{*}P$<0.05 versus indicated groups. APP: amyloid precursor protein; ACR: acrolein; mM: mmol/L; μM: μmol/L.

图5 葡萄糖限制对HT22细胞中阿尔茨海默病相关蛋白的影响

Fig. 5 Effects of GR on AD pathologies-associated proteins in HT22 cells

3　讨 论

到2036年，全球AD新发病例将达到1911.7万例[24]。然而，目前针对中重度AD患者的治疗效果有限，现有疗法仅能改善部分症状，而无法从根本上治疗疾病的病因[25-27]。因此，亟需开发更有效的药物和新型疗法，以实现更理想的疾病管理和风险控制，从而有效缓解医疗压力。

队列研究表明CR与AD和帕金森病发病率降低具有相关性[9,28-29]。动物研究表明，CR可减少β-淀粉样蛋白（amyloid β-protein，Aβ）的沉积从而改善认知功能[30-31]。已有研究证实，葡萄糖作为一种关键的热量来源，对葡萄糖摄入进行限制能延缓衰老[32-35]。目前，细胞水平的热量限制研究主要集中在肿瘤相关领域，多采用80%左右的葡萄糖限制（葡萄糖浓度低至5 mmol/L）[36-38]。认知、衰老、代谢相关的体外热量限制研究相对较少，且不同实验模型中所采用的能量限制方案存在差异，缺乏统一标准。在线虫相关研究中，将线虫培养基葡萄糖从2%（约111 mmol/L）降至0.05%（约2.8 mmol/L，97%限制）持续终生，血糖水平下降60%，线粒体复合体Ⅰ-Ⅳ活性及氧耗率分别提高30%-50%，平均寿命延长25%[39]。将人脐静脉内皮细胞培养基葡萄糖从25 mmol/L降至5 mmol/L（80%限制）维持72 h，可显著增强自噬延缓内皮衰老并促进血管新生[40]。在人神经母细胞瘤细胞系SY5Y亚克隆神经元中，将葡萄糖从25 mmol/L降至5 mmol/L（80%限制）持续48 h，这种长期的80%葡萄糖限制方案使得细胞死亡率升至70%[41]，这一结果似乎也侧面支持本研究中所采用的中等程度葡萄糖限制干预24 h方案。有研究者发现，原代肌卫星细胞采用17.5 mmol/L葡萄糖+减半血清（30%能量限制）处理24 h，细胞的增殖率提高35%，氧化应激水平下降28%，表明适度能量限制可增强干细胞自我更新并减少氧化应激，为30%能量限制干预方案诱导的保护效应提供了直接细胞证据[42]。然而，目前探究GR直接神经保护作用的相关研究缺乏，尤其是其对丙烯醛诱导的海马神经元神经毒性的影响仍然未知。20%~40% CR是目前热量限制实验研究中最常用的方案[21-22]，对于HT22细胞系而言，相较于普通培养基（含25 mmol/L葡萄糖），含17.5 mmol/L葡萄糖的培养基代表30% GR方案。本研究首次对30% GR在缓解丙烯醛诱导的HT22海马神经元神经毒性方面的潜力进行了探索，并进一步揭示了其可能的作用机制，包括减轻氧化应激、抑制细胞凋亡、上调BDNF/TrkB通路以及调节AD相关蛋白（如ADAM-10和BACE-1）的表达。结合本课题组前期在大鼠中的CR相关研究[43]，本研究结果似乎为30% GR对丙烯醛的神经保护作用提供了有力证据。

有研究表明，抗氧化剂可抑制丙烯醛诱导的活性氧（reactive oxygen，ROS）的生成，从而降低其神经毒性[23,44-45]。既往研究还发现，GR可增强细胞对氧化应激和细胞凋亡的抵抗力[33,46]。本研究结果显示，丙烯醛会导致氧化应激水平增加，表现为MDA生成过多、GSH和SOD消耗过多，这与其他研究报道的结果相似[23,47]。有趣的是，采用30% GR方案进行预处理可明显抑制丙烯醛诱导的氧化应激损伤（图2），这表明30% GR的抗氧化特性可能是其神经保护作用的一个重要因素。

抑制细胞凋亡是目前治疗神经退行性疾病（如AD）的策略之一[48-49]。本研究发现，用丙烯醛处理6 h可诱导早期细胞凋亡。此外，为了证实凋亡的发生，本研究还检测了凋亡途径的关键调节因子——Caspase-3/7和Bcl-2。结果显示，丙烯醛降低了Bcl-2的表达，这与之前在其他细胞系或原代神经元细胞中的研究结果一致[50-51]。采用30% GR方案预处理则可增加Bcl-2的表达。本研究还观察到，30% GR方案预处理HT22细胞能显著抑制丙烯醛诱导的Caspase-3/7活性增加。这些结果表明，30% GR可显著抑制丙烯醛诱导的HT22细胞凋亡，表明30% GR在体外具有神经保护作用。

BDNF存在于大脑中，TrkB是其下游受体。BDNF是神经发生和神经元可塑性的重要介质，与长期记忆的形成密切相关[52]。研究表明，CR似乎能上调BDNF表达、神经发生和新生成细胞的存活率，因为它能增强细胞对氧化应激的抵抗力[53]。本课题组前期在大鼠中的研究数据也表明，CR可增加大鼠海马中BDNF/TrkB的表达，同时行为学测试结果也证实CR能够改善丙烯醛引起的认知障碍[43]。同样，本研究进一步证明30% GR可直接改善丙烯醛对海马神经元的神经毒性，而其机制之一与BDNF/TrkB通路的激活有关。然而，在阻断BDNF/TrkB通路的条件下，GR的神经保护作用尚未得到探讨，有待进一步研究。

研究证实,Aβ沉积的病理机制与Aβ生成/清除失衡密切相关[54]。在APP蛋白水解过程中,α-分泌酶(以ADAM10为代表)通过非淀粉样途径裂解APP,减少Aβ生成,发挥神经保护作用;而β-分泌酶BACE1则介导APP在Aβ N末端的裂解,促进Aβ生成[55]。值得注意的是,CR可显著增强α-分泌酶活性,从而减少Aβ沉积[56]。本课题组前期研究发现,CR干预可逆转丙烯醛诱导的BACE1上调和ADAM10下调[43]。本研究进一步证实,30% GR可逆转丙烯醛诱导的海马神经元细胞中BACE-1的升高和ADAM-10的下降,这有力地表明了30% GR方案对治疗AD或延缓AD发病的有益作用。

尽管本研究初步揭示了葡萄糖限制对丙烯醛诱导的神经毒性的保护作用及潜在机制,但仍存在以下几点不足:首先,本研究证实葡萄糖限制可调控BDNF/TrkB信号通路,但并未通过使用TrkB受体拮抗剂或特异性siRNA等手段进行功能丧失性实验,因此无法确立该通路在GR神经保护作用中的必要性;其次,研究所采用的HT22细胞系模型无法完全模拟体内复杂的神经元微环境及疾病进程;再者,本研究主要聚焦于Aβ相关通路,尚未对阿尔茨海默病中另一重要病理标志物——Tau蛋白磷酸化水平进行检测,其在葡萄糖限制干预下的变化仍有待明确。我们需要在后续的研究中,利用原代神经元及动物模型,并结合功能获得与缺失实验,对上述问题进行全面深入的探讨,以期更系统地阐明葡萄糖限制的神经保护机制。此外,本研究中所用的30%葡萄糖限制条件(17.5 mmol/L)是基于HT22细胞常用培养基中葡萄糖浓度进行设置,仍然远高于小鼠或人体生理血糖水平,可能并未模拟"真正的"生理性或轻度低血糖状态,而是模拟了一种从超营养高糖环境向一个仍高于生理水平但相对营养不足环境的转变,在后续的研究中,我们应该同时设置参照小鼠生理血糖水平限制葡萄糖浓度的干预组,观察是否具有类似或更好的神经保护效应。

此外,CR通过整体降低热量摄入发挥效益,其作用机制复杂[22]。本研究选择GR作为切入点,源于葡萄糖是大脑最主要的能量底物,且研究表明,葡萄糖代谢调控是CR的核心下游事件[57-59]。本研究中,30%的GR(17.5 mmol/L)成功模拟了CR的关键表型,如增强抗氧化能力和抗凋亡作用,这提示靶向葡萄糖代谢可能足以介导CR的部分神经保护效应。然而,也必须承认,全身性的CR所涉及的复杂内分泌和系统调节是单纯的细胞GR模型无法完全复现的。未来的研究旨在利用动物模型,验证通过饮食组合(如生酮饮食)或药物干预精准调控大脑葡萄糖代谢的可行性,从而为将CR的益处转化为临床实践提供新策略。

综上所述,本研究数据表明,30% GR对丙烯醛诱导的神经毒性的保护作用是通过抑制氧化损伤、调节BDNF/TrkB通路和APP代谢酶(ADAM-10和BACE-1)实现的。未来需要进一步在BDNF/TrkB通路阻断时评价GR的作用效应变化以及GR对神经退行性疾病其他病理通路的影响。

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