·基础研究·

甲基汞对原代大鼠星形胶质细胞的毒性作用

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[摘要] 目的 初步研究甲基汞对原代 SD 大鼠星形胶质细胞产生毒性的机制。方法 用甲基汞作用于原代 SD 大鼠星形胶质细胞,用高效液相色谱检测细胞氧化还原水平,用 Western-blot 检测核因子 E2 相关因子 2(Nrf2)蛋白表达水平。结果 随着甲基汞浓度的增加,星形胶质细胞的还原水平明显下降。在甲基汞浓度较小时,随着甲基汞浓度的增加,星形胶质细胞 Nrf2 表达水平呈增长趋势;在甲基汞浓度较大时,随着甲基汞浓度的增加,星形胶质细胞 Nrf2 表达水平呈减少趋势。结论 甲基汞对原代 SD 大鼠星形胶质细胞的毒性作用可能通过改变还原型谷胱甘肽(GSH)和 Nrf2 的功能而实现。

[关键词] 甲基汞;星形胶质细胞;毒性作用;核因子 E2 相关因子 2(Nrf2)

Toxicity of Methylmercury on Primary Cultured Rat Astrocyte *CHEN Ru, CAI Ji-yang. Bioscience and Technology School, Tongji University, Shanghai* 200092, *China*

Abstract: Objective To study the mechanism of toxicity of methylmercury on primary cultured SD rat astrocyte. **Methods** Treat primary cultured SD rat astrocyte with methylmercury. Determine the level of cyto-oxidation redoxin with High Performance Liquid Chromatography (HPLC). Western-blot was applied to examine the protein expression of nuclear factor erythroid 2 related factor 2 (Nrf2). **Results** With the increase of the concentration of methylmercury, the redoxin level of astrocyte was apparently decreasing. When the concentration of methylmercury was at a low level, the expression of Nrf2 was increasing with the increase of the methylmercury concentration. When the concentration of methylmercury was at a high level, the expression of Nrf2 was decreasing with the increase of the methylmercury concentration. **Conclusion** The toxicity of methylmercury on primary cultured SD rat astrocyte can be realized through altering the function of GSH and Nrf2.

Key words: methylmercury; astrocyte; toxicity; nuclear factor erythroid-2 related factor 2(Nrf2)

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甲基汞(methylmercury, MeHg)是环境中一种重要的危害人类健康的神经毒素[1]。据研究,甲基汞能在中枢神经系统的星形胶质细胞内积聚,但机制不清。许多研究表明,星形胶质细胞具有潜在介导神经毒的功能[2-6]。转录因子核因子 E2 相关因子 2(nuclear factor erythroid-2 related factor 2, Nrf2)是属于 Cap'n'Collar 家族的转录因子之一,含有碱性亮氨酸拉链(basic region-leucine zipper, bZIP),它在抗氧化反应元件(the antioxidant response element, ARE)介导的抗氧化基因表达中起重要的作用[7-12]。本研究初步探讨了甲基汞对原代 SD 大鼠星形胶质细胞产生毒性的机制。

1 材料和方法

1.1 材料 健康无污染的妊娠雌性 Sprague-Dawley 大鼠:Harlan;MEM 培养基、Earle's 盐、热灭活马血清、FBS、青霉素 G(10000 U/ml)、硫酸链霉素(10000 μg/ml)、dispase 蛋白酶:Invitrogen;Ⅳ型牛胰腺脱氧核糖核酸酶Ⅰ、多聚-L-赖氨酸、sigmacote、Mouse Monoclonal Anti-β-Actin:Sigma;蛋白浓度检测试剂、硝酸纤维素膜:Bio-Rad;丙酮、硼酸、buthionine sulfoximine(BSO)、氯仿、丹磺酰氯、iodoacetic acid、异丙醇、高氯酸、KOH、NaOH、四硼酸钾、$ZnCl_2$、醋酸锌:Sigma-Aldrich(St. Louis, MO);γ-Glutamylglutamate(γ-GG)、oltipraz、sulforaphane、$ZnSO_4$ 分别购自 MP Biomedicals(Irvine, CA)、Rhône-Poulenc Rörer(Lyons, France)、LKT Laboratories Inc.(St. Paul, MN)、Aldrich(Milwaukee, WI);Rabbit Polyclonal anti-Nrf 2、Rabbit Polyclonal anti-GFAP:Santa Cruz Biotechnology,INC;羊抗兔抗体、ECL 化学发光试剂盒:PIERCE;羊抗鼠抗体:Amersham Biosciences;氯化甲基汞(methylmercuric chloride, MeHgCl):ICN Biomedicals(Costa Mesa, CA);HPLC 系统(model 2695 and YMC Pack NH2 Amino column):Waters, Milford, MA。

1.2 方法

1.2.1 原代 SD 大鼠星形胶质细胞的分离与培养 参照 Frangakis 等的方法[13],待妊娠雌性 Sprague-Dawley 大鼠分娩后,取新生 1 d 的幼鼠,断头取脑,取大脑皮层,剥除脑膜,用 dispase 蛋白酶消化,经反复去除消化的脑组织而获得星形胶质细胞,用 MEM 培养基(含有 Earle's 盐、5%热灭活马血清、5% FBS、100 U/ml 青霉素 G、100 μg/ml 硫酸链霉素)将星形胶质细胞接种于 6 孔板,37 ℃、5% CO_2 孵育 24 h,换液以维持星形胶质细胞的贴壁,并去除神经元及少突细胞等杂质,每 3 d 换液 1 次,经星形胶质细胞标记物神经胶质纤维酸性蛋白(glial fibrillary acidic protein, GFAP)的兔源多克隆抗体鉴定,GFAP 阳性细胞大于 95%。

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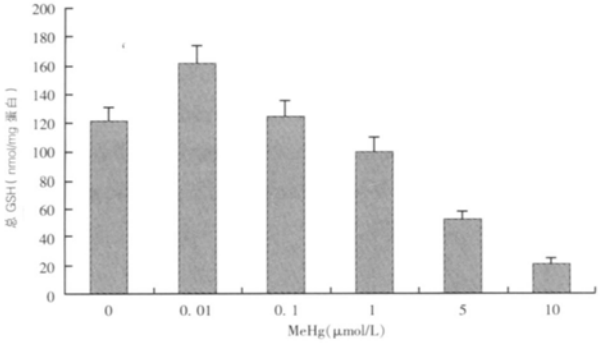
1.2.2 星形胶质细胞氧化还原水平测定　取原代培养 21 d 的星形胶质细胞,用 0、0.01、0.1、1、5、10 μmol/L 甲基汞分别处理,37 ℃、5% CO_2 孵育 6 h。PBS 漂洗,加入冰上预冷的 5% PCA/BA 500 μl,刮下细胞后将细胞悬液转移到离心管中,冰上静置 10 min,4 ℃,13000 g/min 离心 5 min,2 N NaOH 150 μl 溶解离心后的沉淀,用浓度检测试剂检测蛋白浓度。取 300 μl 离心后的上清,加入 7.4 mg/ml 碘乙酸 60 μl,1 N KOH 调节 pH 至 8.8~9.2,室温静置 20 min,加入 20 mg/ml 丹磺酰氯(溶解于丙酮) 300 μl,室温避光静置 24 h,加入 500 μl 氯仿,用高效液相色谱(HPLC)进行检测,记录还原型谷胱甘肽(GSH)、氧化型谷胱甘肽(GSSG)和 γ-GG 的峰参数,分别计算 GSH、GSSG 的浓度,求得总 GSH 浓度。每组 3 次,取平均值。

1.2.3 星形胶质细胞 Nrf2 表达水平测定　取原代培养 21 d 的星形胶质细胞,用 0 μM、0.01 μM、0.1 μM、1 μM、5 μM、10 μM 甲基汞分别处理,37 ℃、5% CO_2 孵育 6 h。经裂解液处理后,进行 10% SDS-PAGE 电泳,电转移至硝酸纤维素膜上,4 ℃封闭过夜,加入 anti-Nrf 2(1∶500) 37 ℃孵育 1 h。TBST 洗涤 3 次,每次 5 min。加入羊抗兔抗体(1∶4000) 37 ℃孵育 1 h。TBST 洗涤 3 次,每次 5 min。ECL 显色,压片,拍照。AlphaEase FC 软件统计各条带的灰度值,计算 Nrf2/actin 比值。

2 结果

2.1 星形胶质细胞氧化还原水平　随着甲基汞浓度的增加,星形胶质细胞的总 GSH 浓度呈下降趋势($P = 7.39 \times 10^{-12}$)。见图 1。



注:总体方差分析,$P = 7.39 \times 10^{-12}$;组间两两比较:0.1 μmol/L 组、1 μmol/L 组与对照组,0.1 μmol/L 组与 0.01 μmol/L 组,1 μmol/L 组与 0.1 μmol/L 组之间均无显著性差异;0.01 μmol/L 组与对照组之间有显著性差异($P < 0.05$);其余各组间比较均有非常显著性差异($P < 0.01$)。

图 1　不同甲基汞浓度下星形胶质细胞的总 GSH 浓度

2.2 星形胶质细胞 Nrf2 表达水平　在甲基汞浓度较小(<1 μmol/L)时,随着甲基汞浓度的增加,星形胶质细胞 Nrf2 表达水平呈增长趋势;在甲基汞浓度较大(>1 μmol/L)时,随着甲基汞浓度的增加,星形胶质细胞 Nrf2 表达水平呈减少趋势。见表 1。

表 1　不同甲基汞浓度下星形胶质细胞 Nrf2 表达水平

甲基汞浓度(μmol/L)	Nrf2/actin 比值
0.0	0.39
0.01	0.41
0.1	0.59
1.0	0.22
5.0	0.62
10.0	0.41

3 讨论

甲基汞是一种存在于环境中的有害微量元素[14],甲基汞首先在水生生物体内蓄积,然后通过食物网进行生物放大到 10000~100000 倍[14-15]。人类暴露于甲基汞的主要途径是通过摄入含甲基汞的食物:成人通过摄入海食品,婴儿通过摄入母乳[16]。在日本和伊拉克进行的许多人类流行病学调查研究表明,甲基汞对人类神经系统有毒性作用[17-19]。研究表明,甲基汞能导致神经系统中某些特定区域的神经损伤,包括人类小脑的粒细胞层以及大脑枕部皮层的距状带[17,20-21]。上述这些特定中枢神经系统区域对甲基汞高度敏感的机制尚未阐明。有研究显示,甲基汞神经毒性的主要机制在于神经元内谷氨酸盐和 γ 氨基丁酸(GABA)的自发性释放[22],以及星形胶质细胞对谷氨酸盐和 GABA 的摄取被抑制[23-24]。

在中枢神经系统中,星形胶质细胞不仅作为填充于神经元间隙的连接性和结构性组织,而且它对维持中枢神经系统的正常功能起到了十分积极的作用。比如星形胶质细胞可产生生长因子,维护细胞的微环境,以及它对突触传递有调节作用等。此外,研究显示,星形胶质细胞具有潜在介导神经毒的功能,这种神经胶质细胞介导的神经元功能损伤可能与星形胶质细胞可以将无毒复合物转化为有毒代谢物的能力有关。比如,作为 1-甲基-4-苯基-1,2,3,6-四氢吡啶(MPTP)神经毒素的毒性代谢产物 MPP^+ 是在星形胶质细胞中形成并从中释放出去而引起神经元损伤[2-6,25]。许多研究表明,星形胶质细胞在介导甲基汞的神经毒性过程中发挥重要作用[26-27]。甲基汞首先在星形胶质细胞中集聚并且抑制谷氨酸盐和半胱氨酸的摄取,这些会破坏 GSH 的合成,并且破坏星形胶质细胞的氧化还原状态[28-32]。分子氧对许多与需氧代谢有关的生物学反应是必需的,这些生物学反应将导致活性氧簇(reactive oxygen species, ROS)的不断形成[33]。许多实验显示,甲基汞诱导了 ROS 的产生[34-40],并且 ROS 可介导甲基汞的神经毒性[34,41-42]。同时也有许多研究表明,某些抗氧化分子如 GSH 前体对甲基汞的神经毒性有保护作用[24,43]。甲基汞对所有的巯基基团有很高的亲和力[44],而 GSH 正是存在于哺乳动物细胞中最主要的富含巯基的三肽。GSH 的主要功能是清除 ROS[45-46]。许多实验表明,在 PC-12 及其他神经细胞系中,甲基汞导致了细胞内 GSH 数量的减少[41,47-49]。

Nrf2 在许多抗氧化酶基因如 HO-1、prxI、thioredoxin-1 等的基础表达和诱导表达过程中发挥重要的调节作用[7,50]。许多研究表明, Nrf2/ ARE 介导的协同和诱导表达作用在细胞抵抗氧化应激和肿瘤形成的过程中发挥重要作用[50-52]。在缺乏 Nrf2 基因的小鼠中,抗氧化酶基因的表达和诱导水平明显降低[53-54]。

本研究显示,低浓度的甲基汞促进了 Nrf2 的表达;当甲基汞的浓度较大时,随着甲基汞浓度的增加,星形胶质细胞 Nrf2 表达水平呈减少趋势。可见高浓度的甲基汞抑制了 Nrf2 的表达,这可能与高浓度的甲基汞对细胞的杀伤作用较强有关。

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