

氯化锂对苯并[a]芘暴露致大鼠胚胎海马神经元损伤的改善作用

张楠^a, 张英英^b, 王鹤霏^a, 陈茜^a, 白文琳^c, 孙谊然^a, 张文平^a

山西医科大学公共卫生学院 a. 卫生毒理学教研室 b. 流行病与卫生统计学教研室 c. 儿少卫生与妇幼保健学教研室, 山西 太原 030000

摘要:

[背景] 诸多研究证实苯并[a]芘 (BaP) 可透过胎盘屏障及血脑屏障引起胚胎神经毒性, 而目前关于外源化合物对 BaP 暴露所致健康损害的干预作用研究鲜有报道。氯化锂 (LiCl) 在临床上广泛应用于情绪调节, 具有神经营养和神经元保护作用。

[目的] 探讨 LiCl 对孕期 BaP 暴露致大鼠胚胎发育及海马神经元损伤的改善作用, 为研究其具体作用机制提供实验依据。

[方法] 10 周龄孕期 SD 大鼠随机分为 5 组, 分别为对照组、植物油组、BaP 组 (20 mg·kg⁻¹ BaP)、LiCl 组 (40 mg·kg⁻¹ LiCl) 及 LiCl 干预组 (20 mg·kg⁻¹ BaP+40 mg·kg⁻¹ LiCl), 每组 6 只。妊娠第 8 天 (GD8) 开始灌胃染毒, 1 次·d⁻¹, 连续 7 d 至妊娠第 14 天 (GD14), 每天记录孕鼠体重。妊娠第 18 天 (GD18) 剖腹取出胎鼠, 记录胎鼠总数、体重、身长及尾长; HE 染色观察胎鼠海马神经元形态; Western blotting 检测胎鼠海马组织糖原合酶激酶-3β (GSK-3β)、pGSK-3β、β-catenin 蛋白表达。

[结果] 实验期间, 对照组、植物油组和 LiCl 组孕鼠体重及胎鼠发育指标差异均无统计学意义 ($P>0.05$)。GD14 和 GD18, BaP 组孕鼠体重 [(323.34±5.71) g、(364.34±10.41) g] 均低于对照组 [(339.17±7.10) g、(390.32±11.35) g] ($P<0.05$), 而 LiCl 干预组孕鼠体重 [(345.05±7.72) g、(398.73±12.32) g] 均高于 BaP 组 ($P<0.05$)。BaP 组胎鼠的体重、身长、尾长分别为 (2.325±0.061) g、(3.072±0.077) cm、(1.135±0.068) cm, 均低于对照组的相应指标值 [(2.563±0.091) g、(3.284±0.078) cm、(1.276±0.041) cm] ($P<0.05$); LiCl 干预组胎鼠上述发育指标值为 (2.669±0.096) g、(3.356±0.107) cm、(1.206±0.041) cm, 均较 BaP 组有上升 ($P<0.05$)。形态学观察发现, BaP 组胎鼠海马组织齿状回区出现神经元皱缩和染色加深, LiCl 干预组有改善。Western blotting 结果显示: 与对照组相比, BaP 组 pGSK-3β 表达降低 (对照组和 BaP 组的灰度值比值分别为 0.87±0.04、0.50±0.04, $F=19.64$, $P<0.001$), GSK-3β 表达升高 (对照组和 BaP 组的灰度值比值分别为 1.53±0.14、2.09±0.11, $F=5.90$, $P=0.011$), β-catenin 表达降低 (对照组和 BaP 组的灰度值比值分别为 1.41±0.14、0.90±0.04, $F=11.16$, $P=0.001$); LiCl 干预可明显逆转由 BaP 暴露引起的 pGSK-3β 表达降低、GSK-3β 表达升高及 β-catenin 表达降低的现象 (灰度值比值分别为: pGSK-3β, 0.69±0.08, $P=0.036$; GSK-3β, 1.59±0.15, $P=0.042$; β-catenin, 1.33±0.08, $P=0.006$)。

[结论] LiCl 干预可改善孕期 BaP 暴露诱导的大鼠胚胎生长发育迟缓及海马神经元损伤, 其机制可能与 LiCl 抑制 GSK-3β 活性进而提高 β-catenin 的表达相关。

关键词: 氯化锂; 苯并[a]芘; 胚胎毒性; 海马神经元; 糖原合酶激酶-3β; β-catenin

Ameliorating effect of lithium chloride on damage of embryonic hippocampal neurons in rats induced by benzo[a]pyrene exposure ZHANG Nan^a, ZHANG Yingying^b, WANG Hefei^a, CHEN Xi^a, BAI Wenlin^c, SUN Yiran^a, ZHANG Wenping^a (a. Department of Health Toxicology b. Department of Epidemiology and Health Statistics c. Department of Child Health and Maternal and Child Health Care, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi 030000, China)

Abstract:

[Background] Many studies have confirmed that benzo[a]pyrene (BaP) can cause embryo neurotoxicity through placental barrier and blood-brain barrier, but there are few reports about the potential interventional effect of xenobiotics on BaP induced health damage. Lithium chloride is

DOI 10.13213/j.cnki.jeom.2021.20501

基金项目

2020 年山西省研究生教育创新项目 (2020SY234); 深圳市瑞普逊干细胞再生医学研究院专项研发项目 (JCJ202003010103)

作者简介

张楠 (1995—), 女, 硕士生;
E-mail: 458135083@qq.com

通信作者

张文平, E-mail: wpzhang@sxmu.edu.cn

伦理审批 已获取

利益冲突 无申报

收稿日期 2020-10-28

录用日期 2021-03-11

文章编号 2095-9982(2021)05-0482-07

中图分类号 R114

文献标志码 A

►引用

张楠, 张英英, 王鹤霏, 等. 氯化锂对苯并[a]芘暴露致大鼠胚胎海马神经元损伤的改善作用[J]. 环境与职业医学, 2021, 38(5): 482-488.

►本文链接

www.jeom.org/article/cn/10.13213/j.cnki.jeom.2021.20501

Funding

This study was funded.

Correspondence to

ZHANG Wenping, E-mail: wpzhang@sxmu.edu.cn

Ethics approval Obtained

Competing interests None declared

Received 2020-10-28

Accepted 2021-03-11

►To cite

ZHANG Nan, ZHANG Yingying, WANG Hefei, et al. Ameliorating effect of lithium chloride on damage of embryonic hippocampal neurons in rats induced by benzo[a]pyrene exposure[J]. Journal of Environmental and Occupational Medicine, 2021, 38(5): 482-488.

►Link to this article

www.jeom.org/article/en/10.13213/j.cnki.jeom.2021.20501

widely used in clinical emotion regulation, with neurotrophic and neuroprotective effects.

[Objective] This animal experiment investigates the ameliorating effect of lithium chloride on the damage of embryonic development and hippocampal neurons in rats exposed to BaP during pregnancy, so as to provide an experimental basis for the study of its specific mechanism.

[Methods] Ten-week-old pregnant SD rats were randomly divided into five groups with six rats in each group: a control group, a soybean oil group, a BaP group ($20\text{ mg}\cdot\text{kg}^{-1}$ BaP), a LiCl group ($40\text{ mg}\cdot\text{kg}^{-1}$ LiCl), and a LiCl intervention group ($20\text{ mg}\cdot\text{kg}^{-1}$ BaP+ $40\text{ mg}\cdot\text{kg}^{-1}$ LiCl). From gestational day 8 (GD8) to GD14, mother rats were administered by gavage once a day for continuous 7 days, and their weights were recorded every day. On GD18, fetal rats were collected from pregnant rats, and the number, weight, body length, and tail length of the pups were recorded. The morphological changes of neurons in hippocampus were observed by HE staining. The expressions of glycogen synthase kinase-3 β (GSK-3 β), pGSK-3 β , and β -catenin in hippocampus were detected by Western blotting.

[Results] During the experiment, the control, soybean oil, and LiCl groups showed no differences in the body weight of pregnant rats and the development indexes of fetal rats ($P>0.05$). On GD14 and GD18, the weights of pregnant rats in the BaP group [(323.34 $\pm$ 5.71) g, (364.34 $\pm$ 10.41) g] were lower than those in the control group [(339.17 $\pm$ 7.10) g, (390.32 $\pm$ 11.35) g] ($P<0.05$), and the weights of pregnant rats in the LiCl intervention group [(345.05 $\pm$ 7.72) g, (398.73 $\pm$ 12.32) g] were higher than those in the BaP group ($P<0.05$). The body weight, body length, and tail length of fetal rats in the BaP group were (2.325 $\pm$ 0.061) g, (3.072 $\pm$ 0.077) cm, and (1.135 $\pm$ 0.068) cm, respectively, which were lower than those in the control group [(2.563 $\pm$ 0.091) g, (3.284 $\pm$ 0.078) cm, and (1.276 $\pm$ 0.041) cm] (all $P<0.05$). The above development indexes of fetal rats in the LiCl intervention group were (2.669 $\pm$ 0.096) g, (3.356 $\pm$ 0.107) cm, and (1.206 $\pm$ 0.041) cm, respectively, which were higher than those in the BaP group ($P<0.05$). The morphological observation results showed that neurons in dentate gyrus of hippocampus in the BaP group shrank and stained deeply, which was improved in the LiCl intervention group. The Western blotting results showed that the expression level of pGSK-3 β in the BaP group was decreased (gray value ratio: 0.87 $\pm$ 0.04 in the control group, and 0.50 $\pm$ 0.04 in the BaP group, $F=19.64$, $P<0.001$), the expression level of GSK-3 β was increased (gray value ratio: 1.53 $\pm$ 0.14 in the control group, and 2.09 $\pm$ 0.11 in the BaP group, $F=5.90$, $P=0.011$), and the expression level of β -catenin was decreased (gray value ratio: 1.41 $\pm$ 0.14 in the control group, and 0.90 $\pm$ 0.04 in the BaP group, $F=11.16$, $P=0.001$). LiCl intervention significantly reversed the decrease of pGSK-3 β and β -catenin expressions and the increase of GSK-3 β expression caused by BaP exposure (grey value for pGSK-3 β was 0.69 $\pm$ 0.08, $P=0.036$; for GSK-3 β was 1.59 $\pm$ 0.15, $P=0.042$; and for β -catenin was 1.33 $\pm$ 0.08, $P=0.006$).

[Conclusion] LiCl intervention can improve embryonic growth retardation and hippocampal neuron damage in rats induced by BaP exposure during pregnancy. The mechanism may be related to the inhibition of GSK-3 β activity and the increase of β -catenin expression by LiCl.

Keywords: lithium chloride; benzo[a]pyrene; embryotoxicity; hippocampal neuron; glycogen synthase kinase-3 β ; β -catenin

苯并[a]芘 (benzo[a]pyrene, BaP) 属于典型的多环芳烃类物质, 广泛存在于化石燃料燃烧, 香烟烟雾及汽车尾气中, 是一种常见的环境致癌物, 可通过呼吸道、消化道等途径进入机体, 引起呼吸系统、心脑血管系统及神经系统疾病等, 严重威胁人体健康^[1]。研究表明 BaP 具有较强的脂溶性, 易透过胎盘屏障及血脑屏障, 引起胎儿出生体重降低, 儿童期智力下降及攻击行为增加等问题^[2-4]。此外, 动物实验研究也发现孕期暴露于 BaP 可导致动物胚胎畸形, 成年认知功能损伤或神经行为缺陷等现象^[5-7]。而目前关于外源化学物对孕期 BaP 暴露所致胚胎神经毒性的干预作用的研究鲜有报道。

近年来, 随着多哈理论 (Developmental Origins of Health and Disease, DOHaD) 即健康和疾病的发育起源的流行, 越来越多的研究人员关注到孕期宫内环境对胎儿生长及出生后长期发育的影响。目前国内外研究发现, 孕期暴露于 BaP 可通过诱导神经细胞过度氧化应激, 线粒体损伤和神经递质活性下降, 细胞凋亡等途径引起神经细胞或突触可塑性损伤, 导致出生缺

陷或出生后神经系统发育异常^[8-9]。Wnt/ β -catenin 是一条进化上保守的信号通路, 对胚胎期和出生后个体生长发育起着至关重要的作用, 具体地说, Wnt/ β -catenin 通路调节神经干细胞的增殖、分化和迁移等关键过程, 对神经系统的发育和动态平衡至关重要, 它的失调与包括神经退行性疾病在内的各种神经系统疾病密切相关^[10]。Marchetti 等^[11]的研究发现, 老年帕金森病患者大脑中的 Wnt/ β -catenin 信号激活可以促进神经再生, 有助于恢复脑室下区微环境和增强神经救援。

氯化锂 (lithium chloride, LiCl) 是临床上广泛使用的情绪稳定剂, 已被证明具有神经营养、促进神经干细胞增殖和抑制神经元凋亡的作用^[12-13]。LiCl 可抑制糖原合酶激酶-3 β (glycogen synthase kinase-3 β , GSK-3 β) 的活性, 主要通过 GSK-3 β 磷酸化失活, 释放其下游蛋白 β -catenin, 使其在细胞核内蓄积, 参与 DNA 转录、翻译, 进而调节下游基因的表达, 促进神经元生长^[14]。而目前, LiCl 对 BaP 暴露所致的胚胎神经毒性是否有改善作用未见报道。因此, 本研究拟构建孕期 BaP 染毒模型, 利用 LiCl 进行干预, 探讨 LiCl 对 BaP 暴露所致

胚胎神经毒性的改善作用,为研究其具体作用机制提供实验依据。

1 对象与方法

1.1 主要试剂及器材

主要试剂:3,4-benzonpyrene (Sigma-Aldrich, 美国),0.9%氯化钠注射液(石家庄四药有限公司,中国),LiCl(上海生工,中国),植物油(金龙鱼公司,新加坡),苏木素-伊红试剂盒、BCA蛋白定量试剂盒、二抗-羊抗兔(博士德,中国),Anti-beta catenin、Anti-GSK-3 β (Abcam, 英国),Anti-pGSK-3 β (Abclonal, 中国)。主要仪器:-80℃超低温冰箱(Thermo, 美国),智能化组织脱水机、石蜡包埋机、全自动切片机(Leica, 德国),超声波震碎仪(新芝生物公司,中国),高速低温离心机(Eppendorf, 德国),酶标仪(Molecular Devices, 美国),电泳仪、化学发光凝胶成像仪(Bio-Rad, 美国)。

1.2 动物染毒及处理

8周龄SPF级SD大鼠[山西医科大学实验动物中心提供,动物生产许可证号:SCXK(晋)2019-0004],饲养于自然节律采光、环境温度(25±1)℃、湿度50%~60%的清洁级环境中,期间自由饮水和进食。适应性饲养2周后,雌雄(1:1)于18:00合笼,次日8:00观察到阴栓视为合笼成功,记为妊娠第0天(GD0),35只雌鼠连续合笼两晚,共合笼成功32只,受孕率为91.4%,从中随机选取30只孕鼠分为5组,每组6只,分别为对照组(Control)、LiCl组(40 mg·kg⁻¹ LiCl)^[15]、植物油组(soybean oil)、BaP组(20 mg·kg⁻¹ BaP)和LiCl干预组(20 mg·kg⁻¹ BaP+40 mg·kg⁻¹ LiCl)。LiCl溶于生理盐水,BaP溶于植物油,GD8开始染毒,每天1次,连续7 d,以每100 g体重0.5 mL体积灌胃的方式给药。LiCl干预处理于BaP染毒前2 h给药,对照组和植物油组给予相同体积的生理盐水或植物油。本项实验研究通过山西医科大学动物福利和伦理委员会审查(批准号:2020GLL042)并严格遵循相关规定。

观察孕鼠染毒期间的一般状态、外观体征、行为活动及摄食情况,记录其体重变化。GD14染毒结束后继续饲养,GD18时对孕鼠麻醉,剖腹取出子宫连胚胎,测量并记录胎鼠总数、体重、身长和尾长,组织保存于-80℃。

1.3 HE染色

取出胚胎后,冰上快速剥离大脑,4%多聚甲醛

固定24 h后,脱水,石蜡包埋,冠状切片厚度为5 μ m;二甲苯常规脱蜡;梯度乙醇水化;37℃苏木素染色5 min;1%的盐酸酒精分化3 s;37℃伊红染液染色20 s;冲洗、脱水、透明、封片;光学显微镜观察并采图。

1.4 Western blotting检测

取出胚胎后,冰上快速剥离海马,加入适量RIPA裂解液、蛋白酶抑制剂及磷酸酶抑制剂,超声30 s,冰上静止15 min,低温离心取上清,BCA法进行蛋白定量,调整蛋白浓度到同一水平,4℃孵育一抗过夜(Anti-beta catenin, 1:5 000; Anti-GSK-3 β , 1:1 000; Anti-pGSK-3 β , 1:2 000),TBST洗3次,4℃孵育二抗(1:5 000)45 min, TBST洗3次, Bio-Rad显影仪显影, Image J 1.46分析蛋白灰度值。

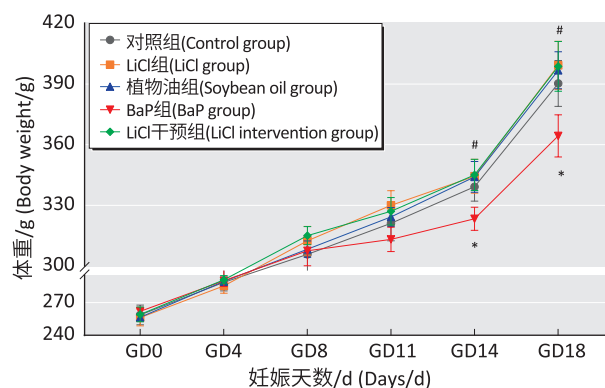
1.5 统计学分析

应用SPSS 20.0和GraphPad Prism 7软件对数据进行统计分析。计量资料采用均数±标准差($\bar{x}$ ±s)表示,服从正态分布且满足方差齐性时,多组间比较用单因素方差分析,多个均数之间两两比较采用Turkey's检验;孕鼠体重变化采用重复测量方差分析,多个均数之间两两比较采用 q 检验。双侧检验水准 $\alpha=0.05$ 。

2 结果

2.1 孕鼠的一般情况及体重变化

染毒期间,各组孕鼠状态良好,外观未见明显异常,被毛光泽。孕鼠体重采用重复测量方差分析,结果如图1所示,各组间孕鼠体重增长不全相同($F_{\text{时间}}=92.25, P<0.001$; $F_{\text{组间}}=3.933, P=0.005$; $F_{\text{交互}}=0.477, P=0.924$)。GD14和GD18,组间主效应显示,植物油



[注]*:与对照组相比, $P<0.05$;#:与BaP组相比, $P<0.05$ 。

[Note]*: Compared with the control group, $P<0.05$; #: Compared with the BaP group, $P<0.05$.

图1 BaP暴露对孕鼠体重增长的影响($n=6$)

Figure 1 Body weight of pregnant rats after BaP exposure ($n=6$)

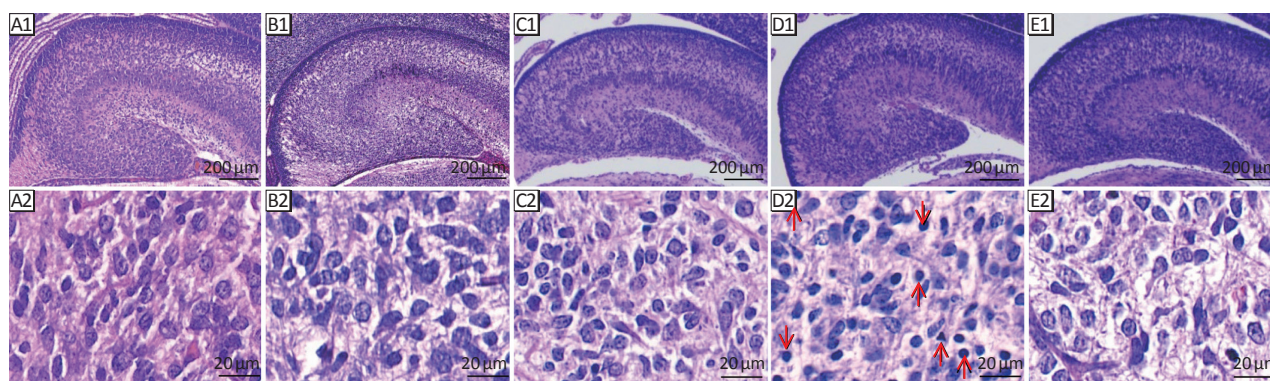
组和LiCl组孕鼠体重增长与对照组相比差异均无统计学意义 (均 $P>0.05$) ; BaP组孕鼠体重 $[(323.34\pm 5.71)\text{ g}$ 、 $(364.34\pm 10.41)\text{ g}]$ 均较对照组 $[(339.17\pm 7.10)\text{ g}$ 、 $(390.32\pm 11.35)\text{ g}]$ 减少, 差异有统计学意义 ($P=0.026$, $P=0.045$) ; LiCl干预组孕鼠体重 $[(345.05\pm 7.72)\text{ g}$ 、 $(398.73\pm 12.32)\text{ g}]$ 较BaP组增加, 差异有统计学意义 ($P=0.034$, $P=0.029$)。

2.2 胎鼠发育情况

表1为GD18胎鼠发育指标测量结果。结果显示, 5组间胎鼠体重、身长、尾长不全相同, 单因素方差分析差异均有统计学意义 ($F_{\text{体重}}=17.60$, $P<0.001$; $F_{\text{身长}}=8.04$, $P<0.001$; $F_{\text{尾长}}=3.38$, $P=0.024$) ; 两两比较, LiCl组和植物油组GD18胎鼠体重、身长、尾长均与对照组差异无统计学意义 (均 $P>0.05$) , BaP组胎鼠体重、身长、尾长均下降, 差异均有统计学意义 ($P<0.001$, $P=0.008$, $P=0.024$) , LiCl干预组胎鼠体重、身长、尾长较BaP组升高, 差异有统计学意义 ($P<0.001$, $P<0.001$, $P=0.022$)。

2.3 胎鼠海马组织的形态学改变

光镜下观察, GD18胎鼠海马组织HE染色结果



[注] A : 对照组 ; B : LiCl组 ; C : 植物油组 ; D : BaP组 ; E : LiCl干预组。1 : $\times 40$ 倍 ; 2 : $\times 400$ 倍。红色箭头代表病理改变, 如海马DG区神经元皱缩, 细胞染色加深。

[Note] A: Control group; B: LiCl group; C: Soybean oil group; D: BaP group; E: LiCl intervention group. 1: $\times 40$; 2: $\times 400$. The red arrows indicate typical pathological changes, including shrinking of neuronal in DG region of hippocampus and deepened cell staining.

图2 BaP暴露对GD18胎鼠海马神经元病理学形态的影响 (HE染色)

Figure 2 Morphology of neurons in hippocampus of GD18 fetal rats after BaP exposure (HE staining)

2.4 胎鼠海马组织pGSK-3 β 、GSK-3 β 及 β -catenin表达水平

与对照组相比, BaP暴露致pGSK-3 β 表达水平降低 (对照组和BaP组的灰度值比值分别为 0.87 ± 0.04 、 0.50 ± 0.04 , $F=19.64$, $P<0.001$) , GSK-3 β 表达升高 (对照组和BaP组的灰度值比值分别为 1.53 ± 0.14 、 2.09 ± 0.11 , $F=5.90$, $P=0.011$) , β -catenin表达降低 (对照组和BaP组的灰度值比值分别为 1.41 ± 0.14 、 0.90 ± 0.04 ,

如图2所示, 对照组、LiCl组和植物油组海马齿状回 (dentate gyrus, DG) 区神经元细胞排列整齐, 形态正常。BaP暴露组出现不同程度神经元胞体缩小, 核固缩, 细胞染色加深, 胞核胞质分界不清, 数量减少, 而LiCl干预组上述情况有所减轻。

表1 BaP暴露对GD18胎鼠发育指标的影响 ($\bar{x}\pm s$)

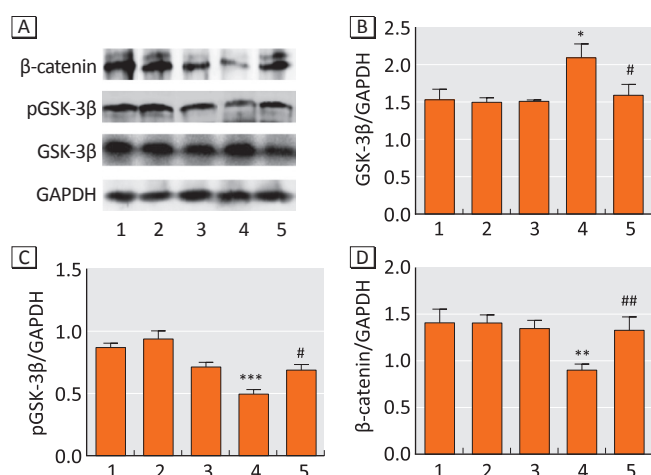
Table 1 Development indexes of GD18 fetal rats after BaP exposure ($\bar{x}\pm s$)

组别 Group	胎鼠数 Number of fetal rats	体重/g Weight/g	身长/cm Body length/cm	尾长/cm Tail length/cm
对照组 (Control)	90	2.563 ± 0.091	3.284 ± 0.078	1.276 ± 0.041
LiCl组 (LiCl)	80	2.678 ± 0.078	3.340 ± 0.086	1.261 ± 0.163
植物油组 (Soybean oil)	80	2.648 ± 0.100	3.281 ± 0.133	1.240 ± 0.090
BaP组 (BaP)	81	$2.325\pm 0.061^{***}$	$3.072\pm 0.077^{**}$	$1.135\pm 0.068^{*}$
LiCl干预组 LiCl intervention	83	$2.669\pm 0.096^{###}$	$3.356\pm 0.107^{###}$	$1.206\pm 0.041^{\#}$
F	—	17.60	8.04	3.38
P	—	<0.001	<0.001	0.024

[注] 与对照组相比, * : $P<0.05$, ** : $P<0.01$, *** : $P<0.001$; 与BaP组相比, # : $P<0.05$, ### : $P<0.001$ 。

[Note] Compared with the control group, * : $P<0.05$, ** : $P<0.01$, *** : $P<0.001$; Compared with the BaP group, # : $P<0.05$, ### : $P<0.001$.

$F=11.16$, $P=0.001$) ; LiCl干预可明显逆转由BaP暴露引起的pGSK-3 β 表达降低、GSK-3 β 表达升高及 β -catenin表达降低的现象 (其灰度值比值分别为 : pGSK-3 β , 0.69 ± 0.08 , $P=0.036$; GSK-3 β , 1.59 ± 0.15 , $P=0.042$; β -catenin, 1.33 ± 0.08 , $P=0.006$)。LiCl组和植物油组的pGSK-3 β 、GSK-3 β 及 β -catenin表达水平与对照组相比差异均无统计学意义。见图3。



[注] A: GSK-3β、pGSK-3β及β-catenin蛋白表达Western blotting电泳图; B~D: GSK-3β、pGSK-3β、β-catenin的Western blotting条带灰度值比值。1: 对照组; 2: LiCl组; 3: 植物油组; 4: BaP组; 5: LiCl干预组。与对照组相比, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$; 与BaP组相比, #: $P < 0.05$, ##: $P < 0.01$ 。

[Note] A: The expressions of GSK-3β, pGSK-3β, and β-catenin measured by Western blotting; B-E: The densitometric expression levels of GSK-3β, pGSK-3β, and β-catenin measured by Western blotting. 1: Control group; 2: LiCl group; 3: Soybean oil group; 4: BaP group; 5: LiCl intervention group. Compared with the control group, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$; Compared with the BaP group, #: $P < 0.05$, ##: $P < 0.01$.

图3 BaP暴露后GD18胎鼠海马组织β-catenin、pGSK-3β及GSK-3β表达水平($n=6$)

Figure 3 Expressions of β-catenin, pGSK-3β, and GSK-3β protein in hippocampus of GD18 fetal rats after exposure to BaP ($n=6$)

3 讨论

日常生活中,人们可以接触到的多环芳烃类物质多种多样,主要来源于有机物的不完全燃烧,如汽车尾气、主/被动吸烟、熏烤食物等^[16]。BaP作为典型的多环芳烃类物质,其致癌性、致畸性和神经毒性众所周知^[17-18]。由于BaP具有较强的脂溶性,易透过胎盘屏障进入胎儿血液中,随血液循环系统透过血脑屏障,影响胎儿神经系统发育^[19-20]。而目前关于外源化合物对BaP孕期暴露所致胚胎毒性的干预作用的研究较少,寻找其预防药物成为国内外关注热点。LiCl是临床上广泛应用的情绪稳定剂,在神经营养、神经元的保护及神经干细胞增殖分化上具有很好的疗效^[14]。以往有研究表明双向情感障碍患者过量或长期的锂治疗可能会存在锂中毒的风险^[21],而细胞实验发现LiCl对大鼠胚胎中脑神经细胞仅具有细胞毒性,而非致畸物^[22],在动物实验中,一定剂量的LiCl对神经退行性疾病具有改善作用^[23]。本研究选取了未对机体产生毒性作用的LiCl剂量进行干预,探讨其对孕期BaP暴露引起的胚胎毒性的保护作用及分子调控机制。

本研究发现,染毒结束后GD14和GD18, BaP组孕鼠体重增长明显下降,表明BaP暴露能够引起胚胎宫内发育迟缓, GD18时BaP组胎儿体重、身长及尾长较对照组减小,证实BaP暴露能够通过慢性毒性蓄积过程对胚胎后期生长发育产生持续影响。刘维春等^[24]在大鼠孕期皮下注射尼古丁GD21取出胎鼠,其身长和尾长均较对照组减小;伦宗姬等^[25]的实验设计大鼠孕前吸入香烟烟雾3个月, GD20麻醉取出胎鼠,发现胎鼠体重、身长、尾长也均较对照组降低。本研究中LiCl干预能够明显改善BaP暴露引起的宫内发育迟缓和胎鼠生长受限,说明LiCl能够缓解BaP暴露导致的胚胎发育迟缓。

脑组织神经元的正常生长是胚胎神经发育的基础。梁婧等^[26]于孕前15d经口给予大鼠BaP ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), GD9时取出胚胎观察胚胎形态,发现胚胎内细胞出现异常凋亡结构等胚胎损伤。本实验利用HE染色技术对海马组织神经元细胞形态进行观察, BaP组胎鼠海马组织神经元细胞呈现部分胞体缩小,核固缩,细胞染色加深,胞核胞质分界不清, LiCl干预能明显减少海马组织神经元固缩,表明孕期BaP暴露可能导致胚胎神经元细胞损伤,而LiCl干预能发挥一定的保护作用。

研究表明LiCl能够通过抑制GSK-3β的活性,进而调控神经干细胞增殖、分化和迁移,增加DG区新生神经元数量^[27-28]。为了阐明LiCl改善BaP暴露导致胚胎神经毒性的分子调控过程,本实验检测了GD18胎鼠海马组织中pGSK-3β、GSK-3β和β-catenin的蛋白表达水平。结果发现与对照组相比, LiCl组pGSK-3β、GSK-3β及β-catenin表达水平差异无统计学意义, LiCl干预组较BaP组pGSK-3β蛋白表达升高, GSK-3β蛋白表达降低及β-catenin蛋白表达升高,提示 $40 \text{ mg} \cdot \text{kg}^{-1}$ LiCl单独作用对GSK-3β的磷酸化作用不大,但与其与BaP联合作用时,可显著改善BaP所致的pGSK-3β表达下降。苗丽萍等^[29]在LiCl对异氟醚诱导发育期幼鼠海马毒性的研究中也发现单独LiCl干预对GSK-3β的磷酸化作用不大,但其可改善异氟醚诱导的幼鼠海马毒性作用。以上结果提示LiCl可通过调控GSK-3β/β-catenin蛋白水平改善BaP暴露所致胚胎毒性及海马组织神经元损伤。本研究通过构建大鼠孕期BaP暴露模型,探讨氯化锂对BaP所致的胚胎神经毒性的改善作用。结果表明,孕期BaP暴露能够引起胚胎生长发育迟缓及海马神经元病理学改变, LiCl对上述形态学损伤有一

定改善作用,其机制可能与 LiCl 抑制 GSK-3 β 活性进而提高 β -catenin 的表达相关。值得深思的是,作为 GSK 的抑制剂,本研究并没有发现单独 LiCl 作用能够引起 pGSK-3 β 表达的升高,这也正是我们需要进一步通过不同干预剂量孕鼠给药或者胎鼠直接给药,从而进行机制方面的深入研究和探讨的关键所在,以期寻求适宜的 LiCl 作用剂量提供科学依据。

参考文献

- [1] BOSTRÖM C E, GERDE P, HANBERG A, et al. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air [J]. *Environ Health Perspect*, 2002, 110 (S3): 451-488.
- [2] LAMICHHANE D K, LEEM J H, KIM H C, et al. Impact of prenatal exposure to polycyclic aromatic hydrocarbons from maternal diet on birth outcomes: a birth cohort study in Korea [J]. *Public Health Nutr*, 2016, 19 (14): 2562-2571.
- [3] 张孝英. 苯并[a]芘暴露对0~6岁儿童智力与行为发育的影响 [D]. 重庆: 重庆医科大学, 2016.
ZHANG X Y. Effects of benzo[a]pyrene exposure on intelligence and behavior development at the age of 0-6 years [D]. Chongqing: Chongqing Medical University, 2016.
- [4] GENKINGER J M, STIGTER L, JEDRYCHOWSKI W, et al. Prenatal polycyclic aromatic hydrocarbon (PAH) exposure, antioxidant levels and behavioral development of children ages 6-9 [J]. *Environ Res*, 2015, 140: 136-144.
- [5] LIN S S, REN A G, WANG L L, et al. Oxidative stress and apoptosis in benzo[a]pyrene-induced neural tube defects [J]. *Free Radic Biol Med*, 2018, 116: 149-158.
- [6] KNECHT A L, TRUONG L, MARVEL S W, et al. Transgenerational inheritance of neurobehavioral and physiological deficits from developmental exposure to benzo[a]pyrene in zebrafish [J]. *Toxicol Appl Pharmacol*, 2017, 329: 148-157.
- [7] MCCALLISTER M M, LI Z, ZHANG T W, et al. Revealing behavioral learning deficit phenotypes subsequent to in utero exposure to benzo(a) pyrene [J]. *Toxicol Sci*, 2016, 149 (1): 42-54.
- [8] CHEN K G, KANG R R, SUN Q, et al. Resveratrol ameliorates disorders of mitochondrial biogenesis and mitophagy in rats continuously exposed to benzo(a) pyrene from embryonic development through adolescence [J]. *Toxicology*, 2020, 442: 152532.
- [9] SLOKOTIN T A, SKAVICUS S, KO A, et al. The developmental neurotoxicity of tobacco smoke can be mimicked by a combination of nicotine and benzo[a]pyrene: effects on cholinergic and serotonergic systems [J]. *Toxicol Sci*, 2019, 167 (1): 293-304.
- [10] SERAFINO A, GIOVANNINI D, ROSSI S, et al. Targeting the Wnt/ β -catenin pathway in neurodegenerative diseases: recent approaches and current challenges [J]. *Expert Opin Drug Discov*, 2020, 15 (7): 803-822.
- [11] MARCHETTI B, TIROLO C, L'EPISCOPO F, et al. Parkinson's disease, aging and adult neurogenesis: Wnt/ β -catenin signalling as the key to unlock the mystery of endogenous brain repair [J]. *Aging Cell*, 2020, 19 (3): e13101.
- [12] SUN Y R, HERRMANN N, SCOTT C J M, et al. Lithium carbonate in a poststroke population: exploratory analyses of neuroanatomical and cognitive outcomes [J]. *J Clin Psychopharmacol*, 2019, 39 (1): 67-71.
- [13] CONTESTABILE A, GRECO B, GHEZZI D, et al. Lithium rescues synaptic plasticity and memory in Down syndrome mice [J]. *J Clin Invest*, 2013, 123 (1): 348-361.
- [14] WEXLER E M, GESCHWIND D H, PALMER T D. Lithium regulates adult hippocampal progenitor development through canonical Wnt pathway activation [J]. *Mol Psychiatry*, 2008, 13 (3): 285-292.
- [15] 宋旭红, 刘继文, 黄贤仪, 等. 锂对小鼠生殖毒性的研究 [J]. *环境与健康杂志*, 1997, 14 (4): 179-180.
SONG X H, LIU J W, HUANG X Y, et al. Research on the reproductive toxicity of lithium in mice [J]. *J Environ Health*, 1997, 14 (4): 179-180.
- [16] YU H Y, LIU Y F, HAN C X, et al. Polycyclic aromatic hydrocarbons in surface waters from the seven main river basins of China: spatial distribution, source apportionment, and potential risk assessment [J]. *Sci Total Environ*, 2021, 752: 141764.
- [17] ZHAO Y, CHEN X M, LIU X Q, et al. Exposure of mice to benzo(a)pyrene impairs endometrial receptivity and reduces the number of implantation sites during early pregnancy [J]. *Food Chem Toxicol*, 2014, 69: 244-251.
- [18] CHEPELEV N L, MOFFAT I D, BOWERS W J, et al. Neurotoxicity may be an overlooked consequence of benzo[a]pyrene exposure that is relevant to human health risk assessment [J]. *Mutat Res Rev Mutat Res*, 2015, 764: 64-89.

- [19] WAKX A, NEDDER M, TOMKIEWICZ-RAULET C, et al. Expression, localization, and activity of the aryl hydrocarbon receptor in the human placenta [J]. *Int J Mol Sci*, 2018, 19 (12) : 3762.
- [20] PATEL B, DAS S K, PATRI M. Neonatal benzo[a]pyrene exposure induces oxidative stress and DNA damage causing neurobehavioural changes during the early adolescence period in rats [J]. *Dev Neurosci*, 2016, 38 (2) : 150-162.
- [21] TOBITA S, SOGAWA R, MURAKAWA T, et al. The importance of monitoring renal function and concomitant medication to avoid toxicity in patients taking lithium [J]. *Int Clin Psychopharmacol*, 2021, 36 (1) : 34-37.
- [22] 侯永根, 端礼荣, 吴全义, 等. 氯化锂对大鼠胚胎中脑神经细胞毒性作用的研究 [J]. *江苏大学学报 (医学版)*, 2003, 13 (4) : 323-324, 326.
- HOU YG, DUAN LR, WU QY, et al. The study of lithium chloride toxicity in rat embryonic midbrain cell *in vitro* [J]. *Acad J Jiangsu Univ (Med)*, 2003, 13 (4) : 323-324, 326.
- [23] PAN YJ, SHORT JL, NEWMAN SA, et al. Cognitive benefits of lithium chloride in APP/PS1 mice are associated with enhanced brain clearance of β -amyloid [J]. *Brain Behav Immun*, 2018, 70 : 36-47.
- [24] 刘维春, 何茹碧, 王婧. 尼古丁对胎儿宫内发育的影响及其甾体激素合成干扰机制 [J]. *广东医学*, 2017, 38 (3) : 362-366.
- LIU WC, HE RB, WANG J. Effect of nicotine on fetal intrauterine development and interference mechanism of steroid hormone synthesis [J]. *Guangdong Med J*, 2017, 38 (3) : 362-366.
- [25] 伦宗姬, 薄其付. 吸入性烟雾对大鼠胚胎发育影响的实验研究 [J]. *中国现代药物应用*, 2011, 5 (5) : 29-30.
- LUN ZJ, BO QF. Experimental study on the effect of inhaling smoke on embryonic development of rats [J]. *Chin J Mod Drug Appl*, 2011, 5 (5) : 29-30.
- [26] 梁婧, 侯海燕, 王梦, 等. 苯并芘的早期妊娠毒性及五味子提取物在胚胎损伤中的保护作用 [J]. *国际生殖健康 / 计划生育杂志*, 2019, 38 (1) : 10-15.
- LIANG J, HOU HY, WANG M, et al. Study on the toxicity of BaP in early pregnancy and protective effect of *Schisandra chinensis* in embryo damage [J]. *J Int Reprod Health/Fam Plann*, 2019, 38 (1) : 10-15.
- [27] PARK J, CHEON W, KIM K. Effects of long-term endurance exercise and lithium treatment on neuroprotective factors in hippocampus of obese rats [J]. *Int J Environ Res Public Health*, 2020, 17 (9) : 3317.
- [28] ALBRECHT LV, TEJEDA-MUÑOZ N, BUI MH, et al. GSK3 inhibits macropinocytosis and lysosomal activity through the Wnt destruction complex machinery [J]. *Cell Rep*, 2020, 32 (4) : 107973.
- [29] 苗利萍, 李珏, 刘雅芳, 等. 氯化锂对异氟醚诱导的发育期幼鼠海马毒性的影响 [J]. *中国药理学杂志*, 2016, 51 (22) : 1923-1928.
- MIAO LP, LI J, LIU YF, et al. Effects of lithium chloride on isoflurane-induced neuroapoptosis in neonatal rat hippocampus [J]. *Chin Pharm J*, 2016, 51 (22) : 1923-1928.

(英文编辑: 汪源; 责任编辑: 丁瑾瑜)