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miR-122-5p、miR-143-3p 及炎症因子 IL-6、IL-10 在双酚 A 和高脂饮食诱导的小鼠非酒精性脂肪性肝病中的表达及意义

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摘要 目的 探究双酚 A(BPA)暴露对 C57BL/6J 小鼠肝脏脂质代谢的影响及作用机制。**方法** 8 周龄雄性 C57BL/6J 小鼠随机分为单纯普通饲料组(ND 组)、普通饲料低剂量 BPA 组(BPA-50 ND 组)、普通饲料高剂量 BPA 组(BPA-500 ND 组)、单纯高脂饲料组(HFD 组)、高脂饲料低剂量 BPA 组(BPA-50 HFD 组)、高脂饲料高剂量 BPA 组(BPA-500 HFD 组),低剂量和高剂量 BPA 组干预剂量分别为 50、500 μg/(kg·d),连续灌胃 12 周。通过 HE 染色分析小鼠肝脏组织变化情况;采用 qRT-PCR 法和 ELISA 法检测肝脏中 miR-122-5p、miR-143-3p 及外周血清白细胞介素(IL)-6 和 IL-10 的表达水平。**结果** HE 染色:ND 组小鼠肝小叶结构完整,其余各组小鼠呈现不同程度的脂滴浸润及肝小叶破坏。随着 BPA 的添加和浓度升高及高脂饮食摄入,外周血 IL-6 浓度逐渐上升,IL-10 浓度逐渐下降。普通饲料组随着 BPA 的添加及浓度加大,miR-122-5p 和 miR-143-3p 表达水平逐渐降低,但高脂饮食组中随着 BPA 的添加及浓度加大,二者表达水平呈逐渐上升趋势。Pearson 相关分析显示:普通饲料组中 miR-122-5p 和 miR-143-3p 表达水平与 IL-10 浓度呈正相关($P<0.01$);高脂饮食组中 miR-122-5p 表达水平与 IL-6 浓度呈正相关($P<0.05$),miR-143-3p 表达水平与 IL-10 浓度呈负相关($P<0.05$)。**结论** 双酚 A 可通过调控 miR-122-5p、miR-143-3p 的表达和调节炎症因子 IL-6、IL-10 的水平诱导非酒精性脂肪性肝病(NAFLD)的发生及进展。

关键词 非酒精性脂肪肝;双酚 A;miR-122-5p;miR-143-3p;炎症因子

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非酒精性脂肪性肝病(non-alcoholic fatty liver disease,NAFLD)是以肝细胞脂肪变性和脂质蓄积为主要特征的代谢性肝损伤。目前全球 NAFLD 的患病率约为 25%,且其发病率呈逐年递增趋势,已成为临床慢性肝病的重要病因[1]。研究[2]表明,摄入过量高脂、高热量食品可引起肝脏游离脂肪酸(free fatty acid,FFA)蓄积,造成肝细胞脂肪变性和脂质蓄积,进而诱发氧化应激和慢性炎症等过程,最终演变为肝纤维化、肝硬化,甚至是肝癌。NAFLD 发病机制复杂,与饮食、遗传、慢性炎症、肠道微生物失调等因素密切相关[3]。目前,除上述因素外,环境因素对 NAFLD 的影响正逐渐被关注。

双酚 A(bisphenol A,BPA)是一种典型的环境内分泌干扰化合物,研究[4]表明,BPA 可诱导脂肪细胞分化、脂质蓄积、炎症及改变体内表观遗传学基因 miRNAs 表达参与 NAFLD 的发生。miRNAs 是非编码 RNA 的一种,在细胞增殖、分化和纤维化等过程中发挥着重要作用。miR-122-5p 和 miR-143-3p 是具有调节肝脏脂质代谢相关基因表达以维持肝脏代谢稳态功能的 miRNAs。研究[5]表明,miR-122-5p 和 miR-143-3p 表达改变可通过调节炎症反应、脂质聚积、肝细胞坏死和肝纤维化等过程参与 NAFLD 的发生发展。而迄今为止国内外关于 BPA 通过调节 miR-122-5p 和 miR-143-3p 表达在 NAFLD 中的研究鲜有报道。该研究通过 BPA 染毒及高脂饲料喂养建立 NAFLD 小鼠模型,探究 BPA 对 NAFLD 小鼠肝脏脂质代谢、表观遗传学和炎症反应的影响。

1 材料与方法

1.1 实验动物 30 只 8 周龄的雄性 SPF 级健康 C57BL/6J 小鼠,平均体质量 21~25 g,购自北京市斯贝福生物科技有限公司,生产许可证号为 SCXK

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(京)2019-0010。小鼠自由进食饮水,环境温度控制在 20 ~ 25 ℃,湿度 55% ~ 65%,自然光照,12 h 光照/12 h 黑暗周期。该实验经过包头医学院动物伦理审查委员会批准(批准号:2023073)。

1.2 试剂与仪器 BPA(上海化成工业发展有限公司,#B0494),玉米油(山东西王食品有限公司),高脂饲料(北京斯贝福生物科技有限公司,#D12492),IL-6 试剂盒(江苏酶免公司,#MM-0163M1)、IL-10 试剂盒(江苏酶免公司,#MM-0176M1),RNA 提取试剂盒(美国 Promega 公司,#LS1040),反转录试剂盒(上海生工生物工程有限公司,#532451)、SYBR Green PCR 试剂盒(北京全式金生物工程有限公司,#AQ601-02),实时荧光定量 PCR 仪(美国 Life Technoiogies 公司),酶标仪(美国 Thermo 公司)。

1.3 模型制备与分组 30 只雄性 C57BL/6J 小鼠随机分为 6 组,单纯普通饲料喂养组(ND 组)、普通饲料低剂量 BPA 组(BPA-50 ND 组)、普通饲料高剂量 BPA 组(BPA-500 ND 组)、单纯高脂饲料组(HFD 组)、高脂饲料低剂量 BPA 组(BPA-50 HFD 组)、高脂饲料高剂量 BPA 组(BPA-500 HFD 组)。ND 组和 HFD 组给予玉米油灌胃,低剂量 BPA 组给予[50 μg/(kg · d)]BPA 灌胃染毒,高剂量 BPA 组给予[500 μg/(kg · d)]BPA 灌胃染毒,持续灌胃 12 周,每周称体质量 1 次。

1.4 样本采集 造模结束后用 2% 的戊巴比妥钠腹腔注射麻醉,麻醉状态下眼球取血,以颈椎脱臼法处死小鼠,立刻取出肝脏组织,称取肝脏质量并计算肝指数,一部分置于 4% 多聚甲醛溶液固定,制备病理切片,一部分迅速放入 -80 ℃中保存。

1.5 ELISA 实验 全血用无菌管收集,室温血液凝固 20 min,4 ℃条件离心 20 min(2 000 r/min)分离血清,于 -80 ℃保存待用。ELISA 试剂盒检测血清 IL-6 和 IL-10 水平,按试剂盒说明书操作。酶标仪检测吸光度值,绘制标准曲线并计算 IL-6 和 IL-10 浓度。

1.6 qRT-PCR 实验 RNA 提取试剂盒提取肝脏样本中总 RNA,紫外分光光度计检测 RNA 浓度和纯度,采用反转录试剂盒将 RNA 反转录为 cDNA,以 cDNA 为模板根据 PerfectStart Green qPCR Super Mix 试剂盒进行聚合酶链扩增反应。引物序列:miR-122-5p (F:5′-GGTGGAGTGTGACAATGGTGTTTG-3′;R:5′-AACGCTTCACGAATTTGCGT-3′);miR-143-3p (F:5′-CGTGAGATGAAGCACTGTAGCTC-3′;R:5′-AACGCTTCACGAATTTGCGT-3′);U6 (F:5′-CTCGCTTCGGCAGCACA-3′;R:5′-AACGCTTCACGAATTTGCGT-3′)。

1.7 统计学处理 采用 GraphPad Prism 8 和 SPSS 25.0 处理实验数据;用 $\bar{x} \pm s$ 表示结果;多组间均数比较采用单因素方差分析,线性相关性分析采用 Pearson 相关分析,$P < 0.05$ 表示差异有统计学意义。

2 结果

2.1 BPA 及高脂饲料诱导的 NAFLD 小鼠外观形态比较 喂养至第 12 周时,普通饲料组小鼠体积大小无明显区别,腰围较细,皮毛细腻;高脂饲料组小鼠较普通饲料组小鼠相比体型肥胖,腰围增粗,皮毛油腻。见图 1。

2.2 BPA及高脂饮食诱导的 NAFLD 小鼠肝脏外观形态变化 普通饲料组中 ND 组小鼠肝脏色泽红

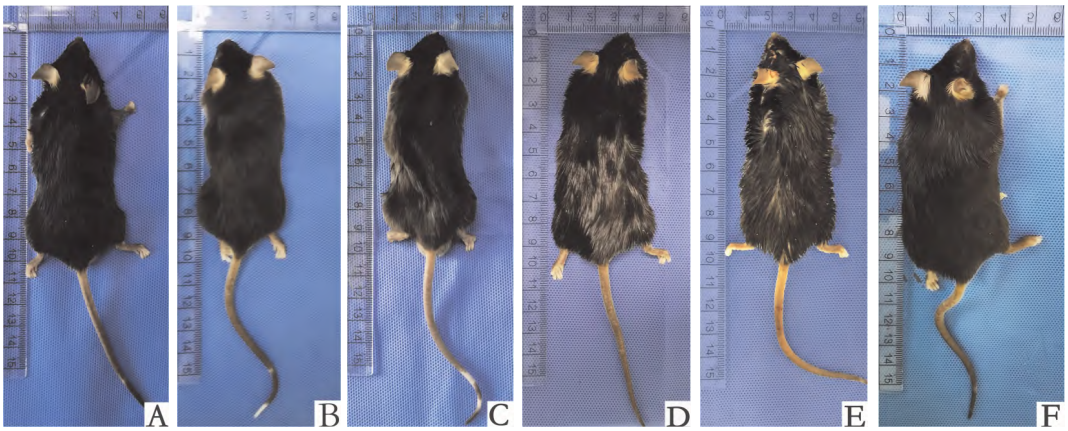

图 1 各组小鼠外观形态

Fig. 1 Appearance and morphology of mice in each group

A - F: ND group, BPA- 50 ND group, BPA- 500 ND group, HFD group, BPA- 50 HFD group, BPA- 500 HFD group.

润鲜亮、边缘锐利;BPA-50 ND、BPA-500 ND 组小鼠与 ND 组小鼠相比肝脏形态较肥厚,颜色较白;高脂饲料组小鼠较普通饲料组相比肝脏体积增大,颜色更白、边缘变钝厚重,表面有油腻感。见图 2。

2.3 BPA 及高脂饲料诱导的 NAFLD 小鼠肝脏 HE 染色结果 ND 组小鼠肝脏肝小叶结构正常,胞质染色均匀,细胞膜完整,界限清楚,细胞充实饱满,肝索围绕中央静脉呈放射状排列;BPA-50 ND 组出现少量脂滴浸润,肝小叶结构正常,伴炎细胞散在浸润;BPA-500 ND、HFD、BPA-50 HFD 组肝脏脂滴细胞数目增多,肝小叶结构欠清晰,肝板内可见纤维组织增生,炎细胞浸润程度加重;BPA-500 HFD 组部分脂滴融合成片,呈蜂窝状或融合成大空泡,肝细胞出现坏死,肝小叶及内部结构消失,肝脏纤维化程度与其余几组相比最为严重。见图 3。

2.4 BPA 及高脂饲料诱导的 NAFLD 小鼠体质量变化情况 随着喂养时间的延长,各组小鼠体质量逐渐上升;高脂饲料组小鼠体质量上升速度显著大于普通饲料组;普通饲料组之间小鼠体质量上升速度无明显变化;高脂饲料组之间小鼠体质量上升速度变化趋势为 BPA-500 HFD 组 > BPA-50 HFD 组 > HFD 组。见图 4。

2.5 各组小鼠体质量、肝脏质量、肝指数比较 根据肝脏指数 = [肝脏质量(g)/体质量(g)] × 100% 计算各组小鼠肝脏指数,结果显示:① 高脂饲料各组小鼠体质量均大于普通饲料组,差异有统计学意义($P < 0.05$);普通饲料组之间小鼠体质量无明显差异;高脂饲料组小鼠随着 BPA 给药浓度的上升,小鼠体质量逐渐变大。② 高脂饲料组小鼠肝脏质量显著大于对应的普通饲料组,差异有统计学意义($P < 0.05$);普通饲料组之间小鼠肝脏质量无明显变化;高脂饲料组小鼠随着 BPA 给药浓度的上升,肝脏质量逐渐变大,且高脂饲料加 BPA 组的肝脏质量大于单纯 HFD 组,差异有统计学意义($P < 0.05$)。③ 高脂饲料加 BPA 组小鼠的肝指数大于普通饲料组($P < 0.05$);普通饲料组之间小鼠肝指数无明显差异;高脂饲料组小鼠随着 BPA 给药浓度的上升,肝指数逐渐变大,其中 BPA-500 HFD 组的肝指数大于单纯 HFD 组,差异有统计学意义($P < 0.05$)。见图 5。

2.6 各组小鼠外周血清 IL-6、IL-10 表达水平 外周血清 IL-6 表达结果显示:随着 BPA 的添加及高脂饲料摄入,IL-6 浓度逐渐上升;普通饲料组 3 组和单纯高脂饲料组(HFD)组共 4 组之间 IL-6 浓度变化不明显;高脂饲料加 BPA 组 IL-6 浓度显著大于上述 4 组,其中 BPA-500 HFD 组小鼠 IL-6 浓度最高,与上述 4 组的差异有统计学意义($P < 0.05$)。外周血清 IL-10 表达结果显示:随着 BPA 的添加及高脂饲

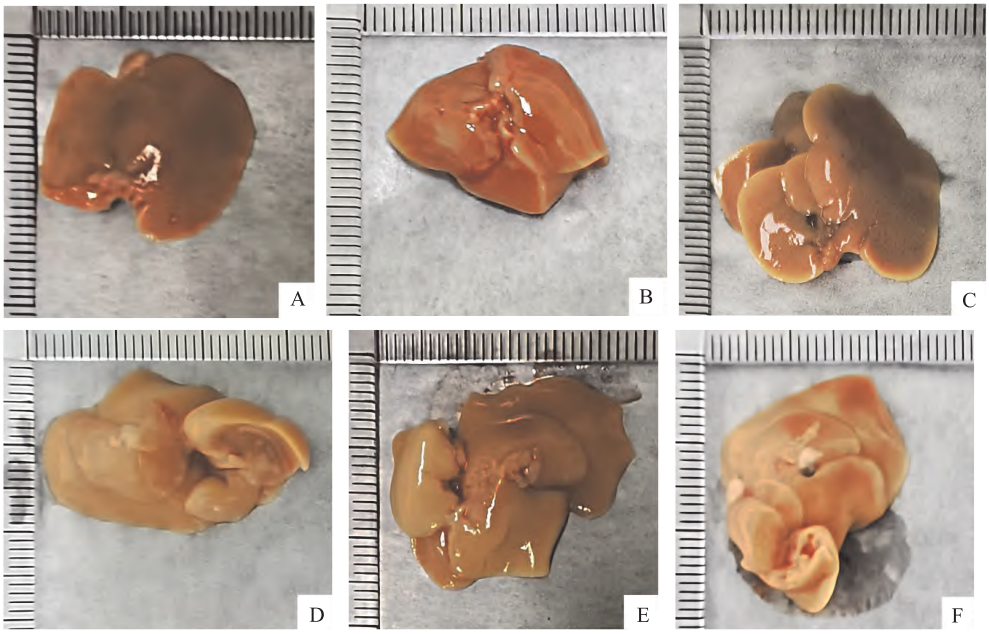

图 2 各组小鼠肝脏外观形态

Fig. 2 The appearance and morphology of mice liver in each group

A – F: ND group, BPA-50 ND group, BPA-500 ND group, HFD group, BPA-50 HFD group, BPA-500 HFD group.

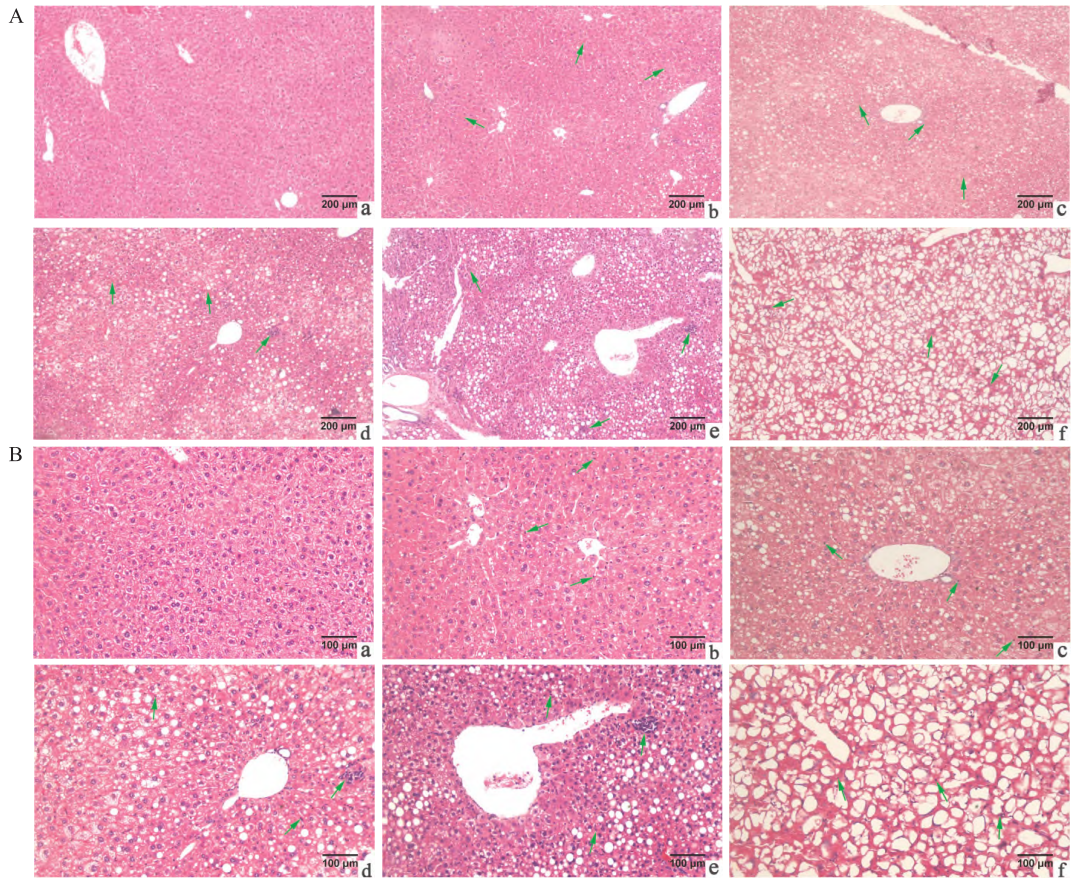

图 3 各组小鼠肝脏 HE 染色

Fig. 3 HE stained liver of mice in each group

A: HE stained liver of mice in each group ×100; B: HE stained liver of mice in each group ×200; a – f: ND group, BPA-50 ND group, BPA-500 ND group, HFD group, BPA-50 HFD group, BPA-500 HFD group; Green arrow: inflammatory cells.

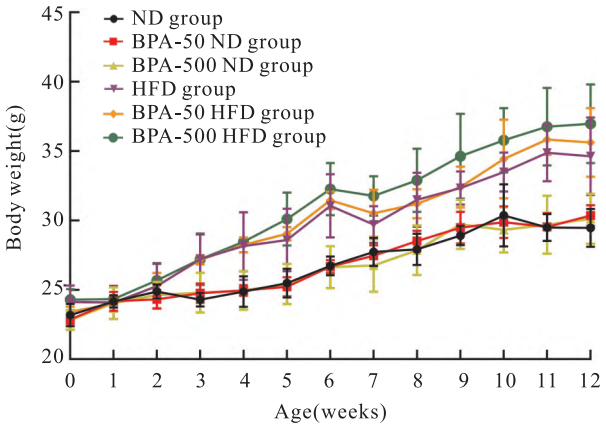

图 4 各组小鼠体质量变化情况

Fig. 4 Changes of body mass of mice in each group

料摄入,IL-10 浓度逐渐下降;ND 组 IL-10 浓度高于其余各组,差异有统计学意义($P<0.05$),BPA-50 ND 组 IL-10 浓度高于 BPA-50 HFD 组和 BPA-500 HFD 组,差异有统计学意义($P<0.05$)。见图 6。

2.7 各组小鼠肝脏中 miR-122-5p、miR-143-3p 表达水平 miR-122-5p 表达结果显示:ND 组 miR-122-5p 表达水平高于其余各组($P<0.05$);普通饲料组随着 BPA 给药浓度的增加,miR-122-5p 表达水平逐渐下降;单纯高脂饲料组 miR-122-5p 表达水平最低,而高脂饲料组给予 BPA 染毒后 miR-122-5p 表达水平逐渐上升。miR-143-3p 表达结果显示:ND 组 miR-143-3p 表达水平高于其余各组,差异有统计学意义($P<0.05$);普通饲料组随着 BPA 浓度的加大,miR-143-3p 表达水平逐渐下降;高脂饲料组随着 BPA 浓度的加大,miR-143-3p 表达水平又逐渐呈上升趋势。见图 7。

2.8 Pearson 相关分析探究 miR-122-5p、miR-143-3p 与 IL-6 和 IL-10 的相关性 普通饲料组中 miR-122-5p 和 miR-143-3p 表达均与 IL-10 浓度呈正相

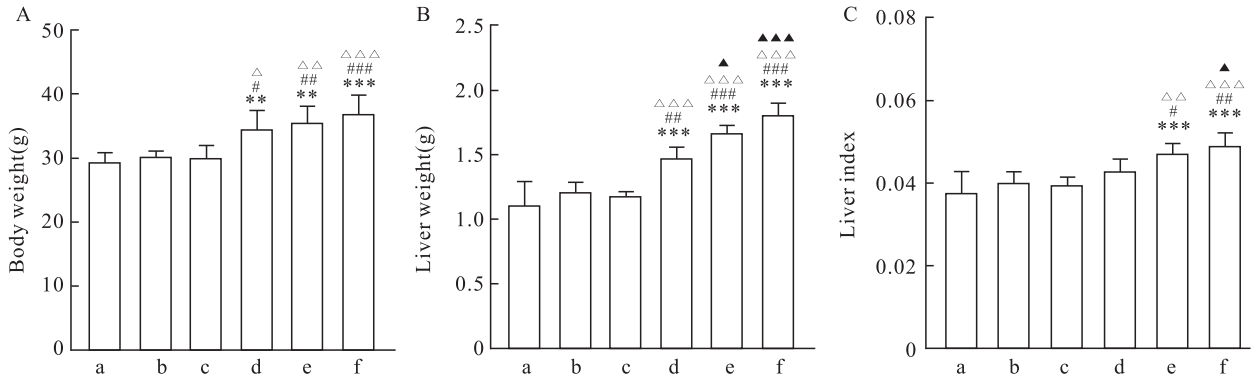

图 5 各组小鼠体质量、肝脏质量和肝指数比较

Fig. 5 Comparison of body mass, liver mass and liver index of mice in each group

A: Comparison of body weight of mice in each group; B: Comparison of liver weight of mice in each group; C: Comparison of liver index of mice in each group; a – f: ND group, BPA-50 ND group, BPA-500 ND group, HFD group, BPA-50 HFD group, BPA-500 HFD group; $^{**}P<0.01$, $^{***}P<0.001$ *vs* ND group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs* BPA-50 ND group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$, $^{\triangle\triangle\triangle}P<0.001$ *vs* BPA-500 ND group; $^{\blacktriangle}P<0.05$, $^{\blacktriangle\blacktriangle\blacktriangle}P<0.001$ *vs* HFD group.

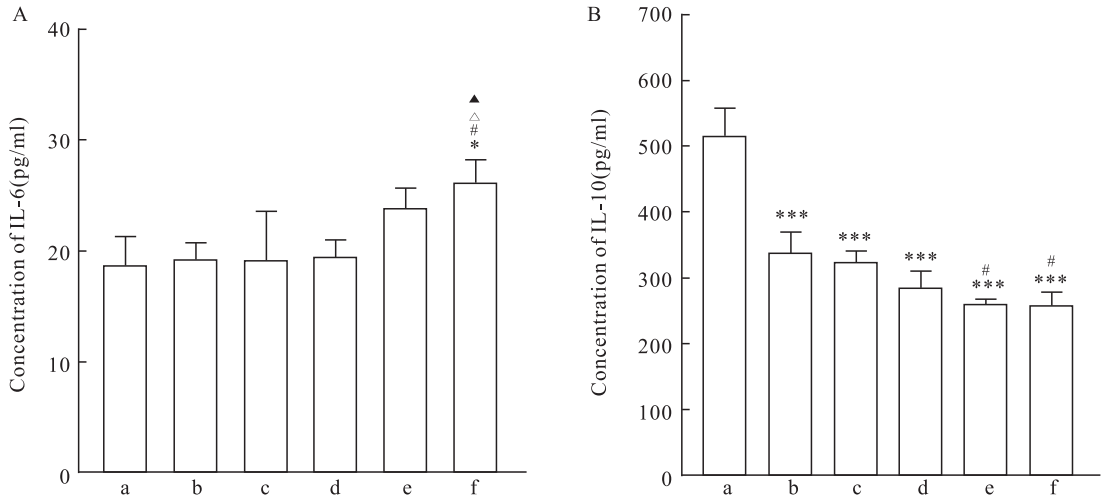

图 6 各组小鼠外周血 IL-6 和 IL-10 表达水平

Fig. 6 Expression levels of IL-6 and IL-10 in peripheral blood of mice in each group

A: Comparison of peripheral blood IL-6 concentration of mice in each group; B: Comparison of peripheral blood IL-10 concentration of mice in each group; a – f: ND group, BPA-50 ND group, BPA-500 ND group, HFD group, BPA-50 HFD group, BPA-500 HFD group; $^{*}P<0.05$, $^{***}P<0.001$ *vs* ND group; $^{\#}P<0.05$ *vs* BPA-50 ND group; $^{\triangle}P<0.05$ *vs* BPA-500 ND group; $^{\blacktriangle}P<0.05$ *vs* HFD group.

关;而与 IL-6 浓度无相关性。高脂饲料组中 miR-122-5p 表达与 IL-6 浓度呈正相关;miR-143-3p 表达与 IL-10 浓度呈负相关。见图 8。

3 讨论

BPA 作为环境内分泌干扰物可通过改变机体正常的代谢途径诱发内分泌代谢紊乱,增加 NAFLD 的发生风险。该研究显示,普通饲料组和高脂饲料组中,随着 BPA 的添加及浓度加大,肝脏脂滴浸润,炎症和纤维化程度逐渐加重。高脂饲料组的上述病理改变大于普通饲料组。因此,BPA 可诱导肝脏脂质聚积、慢性炎症和纤维化反应,且在高脂饮食基础上,BPA 进一步加重了高脂饮食诱导的肝脏脂肪蓄积、炎症和纤维化。研究[6]显示,BPA 可直接调节脂肪生成和分解相关基因表达变化,干扰脂质代谢,诱发氧化应激等过程促进体内脂质沉积。BPA 还可促进炎性因子包括 IL-1β、TNF-α 和 IL-6 等的释放,诱发机体或肝脏局部炎症反应[7]。动物研究[8]显示,BPA 暴露促进了与炎症相关的神经酰胺合成,加速肝脏慢性炎症反应。另有研究[9]表明,BPA

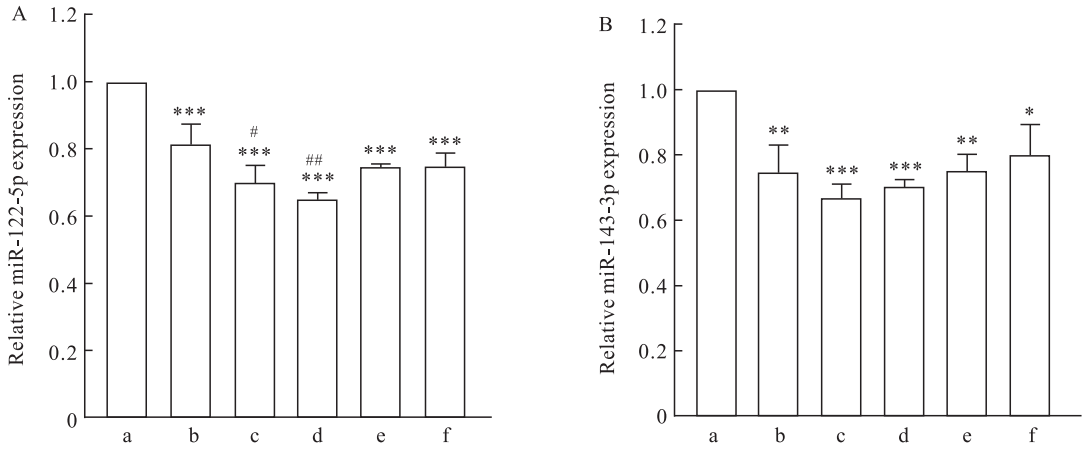

图 7　各组小鼠肝脏中 miR-122-5p 和 miR-143-3p 表达水平

Fig. 7　Expression levels of miR-122-5p and miR-143-3p in liver of mice in each group

A: Comparison of the expression level of miR-122-5p in liver of mice in each group; B: Comparison of the expression level of miR-143-3p in liver of mice in each group; a – f: ND group, BPA-50 ND group, BPA-500 ND group, HFD group, BPA-50 HFD group, BPA-500 HFD group; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* ND group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* BPA-50ND group.

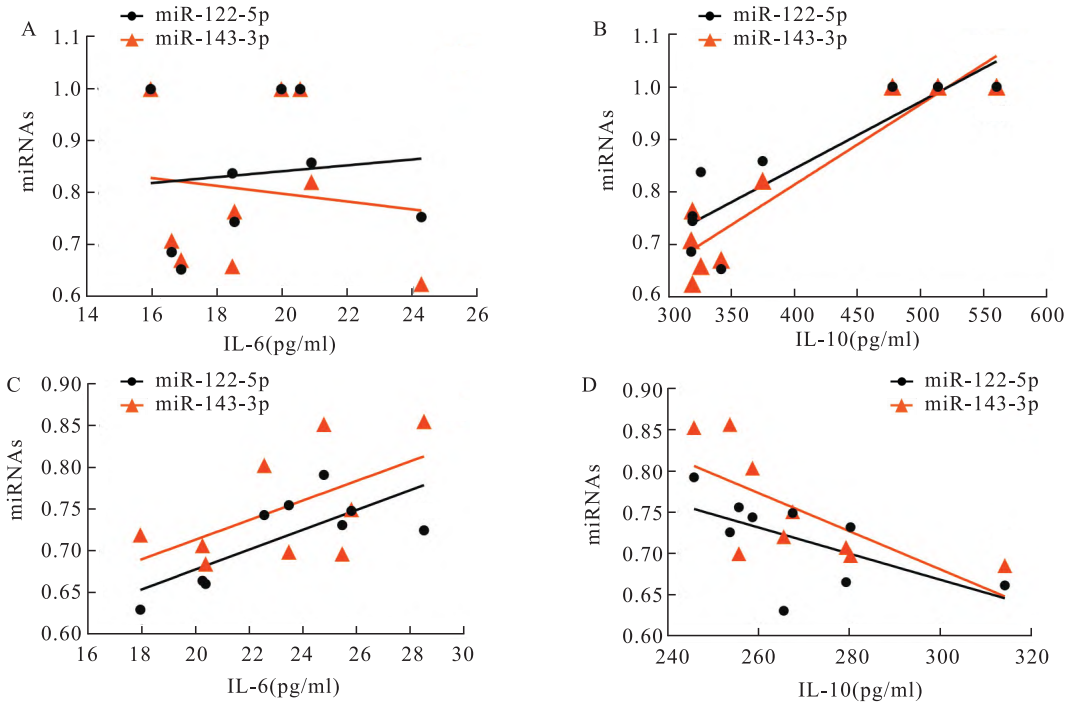

图 8　miR-122-5p 和 miR-143-3p 表达与 IL-6、IL-10 相关性

Fig. 8　Correlation between miR-122-5p and miR-143-3p expression and IL-6, IL-10, respectively

A: Correlation between the expression of miR-122-5p and miR-143-3p and IL-6 in normal diet group; B: Correlation between the expression of miR-122-5p and miR-143-3p and IL-10 in normal diet group; C: Correlation between the expression of miR-122-5p and miR-143-3p and IL-6 in high-fat diet group; D: Correlation between the expression of miR-122-5p and miR-143-3p and IL-10 in high-fat diet group.

与 HFD 协同作用可激活 NLRP3 炎症小体,引起肝脏局部炎症反应和胰岛素抵抗。该研究结果表明,单纯 BPA 和单纯高脂饮食饲养的小鼠所致的早期 NAFLD 中 IL-6 表达与正常对照组相比无明显变化,但此时肝损害仍在持续加重。而在 BPA 与高脂饮食联合喂养下,肝脏脂肪浸润进一步加重导致肝脏纤维化时,IL-6 表达水平显著上升,此时 IL-6 的瀑布式释放表明 NAFLD 处于重症急性炎症反应状态。IL-10 检测结果表明,BPA 和高脂饮食单独作用均可降低 IL-10 表达水平,而当高脂饲料联合 BPA 喂养

小鼠时,NAFLD 已经演变为脂肪性肝炎或肝纤维化,IL-10 表达水平继续降低,提示 IL-10 在 NAFLD 疾病演变过程中一直发挥抗炎及调节组织稳态的作用。

已有较多研究表明 BPA 可诱导 miRNAs 表达改变参与多种疾病的发生发展,如 BPA 可通过抑制 miR-27b-3p 表达水平诱导淋巴细胞凋亡[10]。BPA 还可调节 miR-375、miR-676 和 miR-340-5p 等基因表达,引起体内糖脂代谢紊乱,导致肥胖和糖尿病的发生[11]。但 BPA 是否可以调节 miR-122-5p 和 miR-143-3p 的表达却未可知。该研究结果显示,普通饲料组随着 BPA 暴露及给药浓度的加大,miR-143-3p 和 miR-122-5p 表达水平逐渐下降;而在高脂饮食组中二者表达水平又逐渐呈上升趋势。miRNA-122 是一种具有调节肝脏脂质代谢相关基因表达的非编码 RNA。研究[12]显示,miR-122 表达上升可促进炎症反应、脂质聚积、肝细胞凋亡和肝纤维化,加重肝脏的损伤。相反,另有研究[13]表明,miR-122 表达下降也可引起三酰甘油增加、脂肪聚集和胰岛素抵抗等参与 NAFLD 疾病进展。miRNA-143 也是与脂质稳态密切相关的肝脏特异性 miRNA,但关于其调节脂代谢的作用同样仍存在争议。有研究[14]显示,miR-143 表达上升可降低脂解能力,同时促进脂肪细胞分化和脂肪生成。但有研究[15]指出,miR-143 表达水平降低增加了 NAFLD 患者的肝纤维化风险。在该研究中,普通饲料组即 NAFLD 病变早期,BPA 可通过诱导肝脏脂肪变性下调 miR-122-5p 和 miR-143-3p 表达;而在高脂饲料组即当疾病进展为中晚期即脂肪性肝炎或肝纤维化时,BPA 协同高脂饮食可上调 miR-122-5p 和 miR-143-3p 表达,其原因可能与 BPA 直接或间接诱导强烈的氧化应激和炎症风暴等因素有关。

关于 miRNAs 调节炎症反应参与多种疾病的发生及进展已有较多研究。有研究[16]表明,miR-122-5p 通过靶向 SOCS1/STAT3 信号通路促进 IL-10 释放缓解肝脏炎症反应减轻肝损伤。另有研究[17]表明,miR-143-3p 可抑制 TLR4/MyD88/NF-κB 信号通路促进 IL-10 释放改善支原体肺炎小鼠肺部炎症因子水平。在该研究中,单纯 BPA 喂养导致肝脏轻度脂肪变性时,miR-122-5p、miR-143-3p 与 IL-10 发挥协同作用,抑制肝脏炎症反应和脂肪变性;而 BPA 联合高脂饮食诱导 NAFLD 演变为非酒精性脂肪性肝炎和肝脏纤维化时,miR-122-5p 和 miR-143-3p 通过发挥相反作用促进肝脏的局部炎症反应,加重 NAFLD 小鼠肝脏损伤。

综上所述,BPA 作为公认的环境内分泌干扰物与 NAFLD 的关系密切,该研究表明 BPA 可直接或间接诱发体内炎症反应,并可在不同条件下调节 miRNAs 表达发生特定改变,并且这些改变与 miRNA 特异性、细胞特异性和阶段特异性相关。预防 BPA 造成健康损伤的措施除了减少塑料制品的使用外,还要积极控制体质量,减少肥胖引发的慢性炎症反应,但对于环境中已经存在的 BPA 的影响也不可忽视。因此,今后针对 BPA 引发人体疾病的发病机制进行更为广泛且深入的研究是迫切而必要的。

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Expression and significance of miR-122-5p, miR-143-3p and inflammatory factors IL-6 and IL-10 in mice with nonalcoholic fatty liver disease induced by bisphenol A and high-fat diet

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Abstract ***Objective*** To explore the impact of bisphenol A (BPA) exposure on liver lipid metabolism in C57BL/6J mice and uncover the mechanisms at work. ***Methods*** Male C57BL/6J mice, aged eight weeks, were stratified into six cohorts: a control group on a standard diet (ND), a group on a standard diet with low-dose BPA (BPA-50 ND), a group on a standard diet with high-dose BPA (BPA-500 ND), a control groupon a high-fat diet (HFD), a group on a high-fat diet with low-dose BPA (BPA-50 HFD), and a group on a high-fat diet with high-dose BPA (BPA-500 HFD). Dosages for the low- and high-dose BPA groups were 50 and 500 μg/(kg · d), respectively, administered *via* gavage over a duration of 12 weeks. Hepatic tissue underwent histological examination through hematoxylin and eosin (HE) staining. Furthermore, the expression levels of miR-122-5p and miR-143-3p in hepatic tissue, in addition to interleukin (IL)-6 and IL-10 in peripheral serum, were quantitatively measured employing quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. ***Results*** Histopathological analysis *via* HE staining indicated intact hepatic lobule architecture in the ND group, whereas other groups displayed variable degrees of lipid droplet accumulation and damage to hepatic lobules. Notably, supplementation with BPA, particularly in conjunction with a high-fat diet, led to a progressive increase in IL-6 levels and a decrease in IL-10 levels in peripheral blood. In the context of a standard

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exposed to 25 mmol/L D-glucose for simulating an *in vitro* hyperglycemic (HG) environment. The control group was exposed to a 20 mmol/L mannitol + 5.5 mmol/L glucose environment. Rats were randomly divided into normal control group, DR group, and DR + Gas6 group, with 20 rats in each group. A DR model was established by intraperitoneal injection of STZ solution. Cell proliferation was evaluated using the cell count kit 8(CCK-8) assay. Lipid reactive oxygen species (ROS) levels were measured by flow cytometry, and levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were measured by biochemical assays to evaluate iron death. The expression of Gas6 and MerTK proteins was analyzed by Western blot. ***Results*** Compared with HG group, the cell viability, SOD, GSH-Px levels in HG + Gas6 group increased significantly ($P < 0.05$), while the levels of lipid-ROS and MDA decreased significantly ($P < 0.05$). In HG + sh-Gas6 group, the cell viability, SOD and GSH-Px levels decreased significantly ($P < 0.05$), while the levels of lipid-ROS and MDA increased significantly ($P < 0.05$). In addition, the expression of GPX4 protein in HG + Gas6 group was significantly higher than that in HG group ($P < 0.05$), and the expression of GPX4 protein in HG + sh-Gas6 group was significantly lower than that in HG group ($P < 0.05$). Compared with the control group, the average thickness of retinal nerve fiber layer in DR group significantly decreased ($P < 0.05$), while that in DR + Gas6 group increased significantly ($P < 0.05$). In addition, the levels of MDA and iron in retinal pigment epithelium (RPE) tissues of DR + Gas6 group decreased significantly ($P < 0.05$), while the levels of GSH and the expressions of Gas6, MerTK and GPX4 proteins increased significantly ($P < 0.05$). ***Conclusion*** HG treatment accelerates the clearance of GPX4 by inhibiting the Gas6/MerTK signaling pathway, inducing ferroptosis and cell growth inhibition in ARPE-19 cells. In addition, up-regulating the expression of Gas6/MerTK signal in DR rat retina can alleviate ferroptosis and oxidative stress in RPE tissue, and help to restore the average retinal nerve fiber layer thickness.

Key words diabetic retinopathy; growth arrest-specific 6; Mer tyrosine kinase; ferroptosis; rat

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diet, an augmentation in BPA concentration corresponded with a decline in the expression of miR-122-5p and miR-143-3p. Conversely, within the high-fat diet cohort, enhanced BPA concentrations were associated with increased expressions of these microRNAs. Pearson correlation analysis disclosed a significant positive correlation between the expression of miR-122-5p and miR-143-3p and the level of IL-10 in the standard diet group ($P < 0.01$). In the high fat diet group, the expression level of miR-122-5p was positively correlated with the concentration of IL-6 ($P < 0.05$), and the expression level of miR-143-3p was negatively correlated with the concentration of IL-10 ($P < 0.05$). ***Conclusion*** BPA can induce the occurrence and progression of nonalcoholic fatty liver disease (NAFLD) by regulating the expression of miR-122-5p and miR-143-3p and regulating the levels of inflammatory factors IL-6 and IL-10.

Key words nonalcoholic fatty liver disease; bisphenol A; miR-122-5p; miR-143-3p; inflammatory factors

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