

蓝光照射对小鼠视网膜形态和功能的损伤作用

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【摘要】 目的 通过观察蓝光照射对 C57BL/6J 小鼠视网膜形态和功能的影响,探讨非渗出性年龄相关性黄斑变性(AMD)的模型。方法 采用投币法将 20 只 8 周龄清洁级 C57BL/6J 雄性小鼠随机分为正常对照组和蓝光照射组。蓝光照射组小鼠暗适应 24 h 后暴露于 10 000 lx 蓝光下 5 d,正常对照组小鼠按 12 h/12 h 正常光照/黑暗的周期饲养于正常光照环境 5 d。采用光相干断层扫描成像(OCT)活体检查各组小鼠视网膜厚度变化,采用视网膜电图(ERG)检查各组小鼠视网膜功能变化。于光照结束后 24 h 采用颈椎脱臼法处死小鼠并制备眼球壁标本,采用免疫荧光染色法测定小鼠视网膜中视紫红质(Rho)、紧密连接蛋白(ZO-1)和 β -catenin 蛋白表达。结果 蓝光照射组小鼠视网膜上部和下部距视盘 200、400、600、800 和 1 000 μm 处视网膜外核层厚度均较正常对照组变薄,差异均有统计学意义(均 $P < 0.05$)。蓝光照射组小鼠暗适应和明适应 b 波振幅分别为 $(305.50 \pm 41.52) \mu\text{V}$ 和 $(119.50 \pm 6.67) \mu\text{V}$,分别低于正常对照组的 $(415.50 \pm 28.77) \mu\text{V}$ 和 $(139.75 \pm 8.26) \mu\text{V}$,差异均有统计学意义(均 $P < 0.05$)。正常对照组小鼠 RPE 细胞呈正六边形,视网膜各层形态规则,Rho、ZO-1 和 β -catenin 荧光较强;蓝光照射组小鼠 RPE 细胞形态不规则,ZO-1 染色减弱或消失, β -catenin 染色和 Rho 蛋白荧光强度减弱。结论 蓝光照射小鼠视网膜变薄,视网膜功能减弱。

【关键词】 光/不良作用; 视网膜/辐射效应; 视网膜/病理; 光相干断层扫描成像; 视网膜电图; 免疫荧光技术; 近交系 C57BL 小鼠

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Damage of blue-light exposure to retinal morphology and function in mice

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【Abstract】 **Objective** To study the damage effects of blue-light exposure on retinal morphology and function in mouse. **Methods** Twenty 8-week-old clean C57BL/6J mice were randomly divided into blue-light exposure group and normal control group by coin tossing method. The mice in the blue-light exposure group was exposed to 10 000 lx blue light for 5 days after dark adaptation for 24 hours, and the mice in the normal control group was kept under the normal light intensity for 5 days at 12-hour light/12-hour darkness cycles. The retinal thickness was detected by optical coherence tomography (OCT), and retinal function was evaluated by electroretinogram (ERG). The mice was sacrificed and the frozen section and flat mount of eyeball wall was created at 24 hours after irradiation. The expressions of rhodopsin (Rho), zonula occludens-1 (ZO-1) and β -catenin in the retinas were detected by immunofluorescent staining. The use and care of the experimental animals adhered to ARVO Statement by American Society of Visual and Ophthalmological Sciences (No. IACUC-1803029). **Results** The thickness of the retinal outer nuclear layer at 200, 400, 600, 800 and 1 000 μm from the superior and inferior to optic nerves were thinned in the mice of the blue-light exposure group compared with those of the normal control group, showing significant differences between the two groups (all at $P < 0.05$). The b-wave amplitude of the scotopic and photopic ERG was $(305.50 \pm 41.52) \mu\text{V}$ and $(119.50 \pm 6.67) \mu\text{V}$ in the blue-light exposure group, respectively, which was significantly reduced in comparison with $(415.50 \pm 28.77) \mu\text{V}$ and $(139.75 \pm 8.26) \mu\text{V}$ of the normal control group (both at $P < 0.05$). Immunofluorescent staining showed that the retinal pigment epithelial (RPE) cells of the mice exhibited hexagonal shape with regular arrangement, retinal morphology was regular, and the expressions of Rho, ZO-1 and β -catenin proteins showed stronger fluorescence in the retinas of normal control group. However, structural disorder, diminishing fluorescence intensity of Rho, ZO-1 and β -catenin were found in the blue-light exposure group. The

检查。小鼠暗适应后在微弱红色光源的暗室中采用 $0.025 \text{ cd} \cdot \text{s}/\text{m}^2 (-1.6 \log \text{ cd} \cdot \text{s}/\text{m}^2)$ 的刺激光记录暗视 ERG, 平均记录 10 次, 刺激间隔为 30 s。暗适应 ERG 记录后明适应 10 min, 采用 $0.4 \log \text{ cd} \cdot \text{s}/\text{m}^2$ 的白光进行刺激, 记录明视, 分析暗视和明视 ERG b 波振幅及其潜伏值。采用 $30 \text{ cd}/\text{m}^2$ 曝光 10 min, 在 510 nm 和 360 nm 波长处记录 M-视锥和 S-视锥 ERG 反应。采用 $25 \text{ cd} \cdot \text{s}/\text{m}^2 (1.4 \log \text{ cd} \cdot \text{s}/\text{m}^2)$ 刺激光进行刺激, 记录 M-视锥 ERG 反应, 以 $2.5 \text{ cd} \cdot \text{s}/\text{m}^2 (0.4 \log \text{ cd} \cdot \text{s}/\text{m}^2)$ 刺激光进行刺激, 记录 S-视锥 ERG 反应, 每个刺激光强度平均记录 25 个反应, 导出数据后测量 b 波振幅和潜伏期。

1.2.4 免疫荧光染色法检测小鼠视网膜中 Rho 表达

各组于照射后 24 h 用颈椎脱臼法处死小鼠, 摘除双侧眼球, 剪除角膜及虹膜, 将眼球置于 4% 多聚甲醛 4 ℃ 过夜。去除标本中晶状体后用质量分数 10%、20% 和 30% 的蔗糖溶液梯度脱水, OCT 包埋, -80 ℃ 液氮中速冻保存, 制备 10 μm 冰冻切片。将冰冻切片置于质量分数 0.3% Triton X-100 和体积分数 5% BSA 孵育 1 h, 5% BSA 稀释 rhodopsin 一抗 (1:200) 和 PNA (1:200), 4 ℃ 孵育过夜, 1 倍磷酸盐缓冲液 (phosphate buffered solution, PBS) 洗 3 次, 每次 10 min, 1 倍 PBS 稀释二抗 (1:500), 浸入二抗混合物后, 用 DAPI (1:1 000) 染色 10 min。PBS 洗涤 3 次, 盖玻片和封片剂封片, 4 ℃ 条件下避光保存, 激光共聚焦电子显微镜拍照。

1.2.5 荧光染色法检测视网膜铺片中 ZO-1 和 β-catenin 表达

颈椎脱臼法处死小鼠, 摘除双侧眼球, 剪除角膜及虹膜后置于 4% 多聚甲醛 4 ℃ 过夜, 移除晶状体和神经视网膜, 将 RPE、脉络膜和巩膜剪成 8 片置于 EP 管中。PBS 中洗涤平铺片, 室温下用 0.3% Triton X-100 和 5% BSA 封闭 1 h。PBS 洗涤 3 次, 在兔抗 ZO-1 抗体 (1:100, 61-7300) 和兔抗 β-catenin 抗体 (1:200, ab32572) 中 4 ℃ 孵育过夜。PBS 洗涤 3 次, 将平铺片与 Alexa Fluor 488 山羊抗兔 IgG 抗体 (H + L) (1:500) 在室温下孵育 2 h。PBS 洗涤 3 次, 平铺片固定在载玻片上, 盖玻片和封片剂封片, 4 ℃ 条件下避光保存, 激光共聚焦电子显微镜拍照。

1.3 统计学方法

采用 SPSS 20 统计学软件进行统计分析。本研究检测指标的计量资料经 W 检验呈正态分布, 以 mean ± SD 表示, 组间数据经 Levene 检验方差齐。采用完全随机分组单因素干预两水平实验设计, 正常对照组与蓝光照射组间小鼠视网膜外核层厚度差异比较以及视网膜暗视和明视 ERG b 波振幅差异比较均采用独立

样本 t 检验。P < 0.05 为差异有统计学意义。

2 结果

2.1 各组小鼠视网膜外核层厚度比较

与正常对照组比较, 蓝光照射组小鼠视网膜外核层厚度变薄 (图 1)。蓝光照射组小鼠视网膜上部和下部距视盘 200、400、600、800 和 1 000 μm 处视网膜外核层厚度较正常对照组变薄, 差异均有统计学意义 (均 P < 0.05) (表 1)。

2.2 两组小鼠 ERG 波形比较

蓝光照射组小鼠暗视和明视 ERG b 波振幅低于正常对照组, 差异均有统计学意义 (暗视: $t = 4.356, P = 0.005$; 明视: $t = 3.817, P = 0.009$) (表 2, 图 2)。

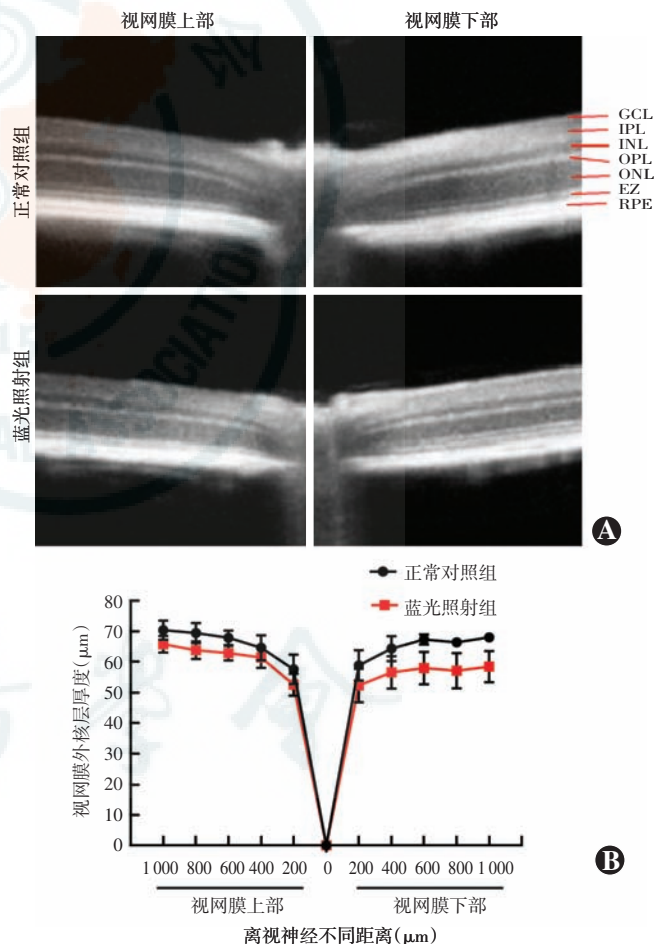


图 1 各组小鼠视网膜外核层厚度比较 A: 正常对照组与蓝光照射组小鼠视网膜 OCT 表现 B: 2 组小鼠视网膜外核层厚度比较 GCL: 神经节细胞层; IPL: 内丛状层; INL: 内核层; OPL: 外丛状层; ONL: 外核层; EZ: 椭圆体带; RPE: 视网膜色素上皮层 Figure 1 Comparison of mouse retinal ONL thickness between two groups A: Retinal OCT performance of mice between two groups B: Comparison of mouse retina ONL thickness in two groups GCL: ganglion cell layer; IPL: inner plexiform layer; INL: retinal inner nuclear layer; OPL: outer plexiform layer; ONL: retinal outer nuclear layer; EZ: ellipsoid region; RPE: retinal pigment epithelium

表 1 两组小鼠视网膜外核层厚度比较 (mean±SD, μm)
Table 1 Comparison of mouse retina outer nuclear layer thickness between two groups (mean±SD, μm)

组别	样本量	距视盘经不同距离的外核层厚度(μm)										
		上部距视盘不同部位视网膜厚度					视盘	下部距视盘不同部位视网膜厚度				
		1 000	800	600	400	200	0	200	400	600	800	1 000
正常对照组	8	70.32±3.15	69.33±3.23	67.75±2.43	64.53±4.08	57.48±4.84	0	58.76±4.96	64.29±4.12	67.25±1.65	66.30±1.58	67.89±1.31
蓝光照射组	8	65.68±2.73	63.74±2.85	62.79±2.38	61.35±3.40	52.47±3.59	0	52.22±5.49	56.50±5.33	57.89±5.31	57.02±5.78	58.38±5.11
<i>t</i> 值		3.149	3.673	4.13	1.693	2.354		2.506	3.269	4.757	4.386	5.104
<i>P</i> 值		0.007	0.003	0.001	0.113	0.034		0.025	0.006	<0.01	<0.01	<0.01

注:(独立样本 *t* 检验)
 Note:(independent simple *t*-test)

表 2 两组小鼠 ERG b 波振幅比较 (mean±SD, μV)
Table 2 Comparison of ERG b-wave amplitude of mice between two groups (mean±SD, μV)

组别	样本量	暗视 b 波振幅	明视 b 波振幅
正常对照组	4	415.50±28.77	139.75±8.26
蓝光照射组	4	305.50±41.52	119.50±6.67
<i>t</i> 值		4.356	3.817
<i>P</i> 值		0.005	0.009

注:(独立样本 *t* 检验) ERG:视网膜电图
 Note:(independent-samples *t* test) ERG:electroretinogram

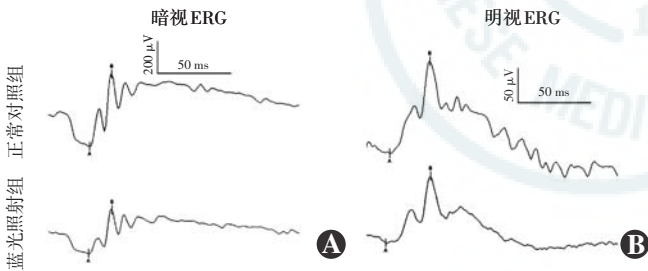


图 2 各组小鼠 ERG 表现 A:2 个组小鼠暗视 ERG b 波振幅 B:2 个组小鼠明视 ERG b 波振幅 ERG:视网膜电图
Figure 2 ERG performance of mice between two groups A: Scotopic ERG b wave amplitude B:Photopic ERG b wave amplitude ERG:electroretinogram

2.3 两组小鼠视网膜 Rho 表达比较

免疫荧光检测显示,正常对照组小鼠视网膜各层形态规则,细胞排列整齐,PNA 和 Rho 共定位显示视网膜中 Rho 呈红色强荧光,外核层可见蓝色强荧光,视网膜厚度正常。蓝光照射组小鼠视网膜中 Rho 红色荧光较正常对照组减弱,视网膜厚度明显变薄,结构紊乱(图 3)。

2.4 两组小鼠 RPE 中 ZO-1 和 β-catenin 蛋白表达比较

正常对照组小鼠 RPE 细胞呈正六边形,ZO-1 和 β-catenin 呈绿色荧光,边界清晰,细胞核呈蓝色荧光,细胞排列较规则。蓝光照射组小鼠 RPE 细胞 ZO-1 和

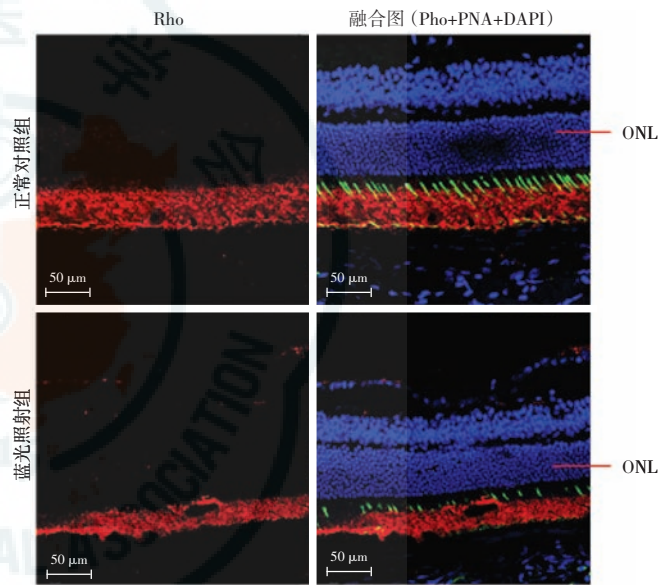


图 3 小鼠视网膜各层冰冻切片中 Rho 表达 (标尺 = 50 μm) 免疫荧光染色显示视网膜中 Rho 表达呈红色荧光,PNA 表达呈绿色荧光,细胞核呈蓝色荧光。与正常对照组相比,蓝光照射组小鼠 Rho 荧光减弱 ONL:视网膜外核层;Rho:视紫红质;PNA:花生凝集素
Figure 3 Immunofluorescence staining of expression of Rho in mouse retina (bar = 50 μm) The expression of Rho showed the red fluorescence,and PNA expression showed the green fluorescence,and the cellular nuclei was blue staining. Compared with the normal control group,the expression of Rho in the mouse retinas of blue-light exposure group was weakened ONL: outer nuclear layer; Rho: rhodopsin; PNA: Peanut agglutinin

β-catenin 荧光减弱或消失,边界不清,细胞核荧光减弱,细胞形态不规则(图 4)。

3 讨论

光损伤引起的视网膜病变可分为两大类,一类为最低光能量所致的病变,临床表现为轻度 RPE 损害,晚期可能有光感受器损伤;另一类为较高能量的损伤,RPE 及视网膜神经上皮均有不同程度损害。线粒体凋亡通路参与蓝光照射所致的 RPE 细胞凋亡过程^[16],同时 ROS 抑制 RPE 细胞对摄入的杆状体外段

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稿件审理过程中作者可通过“中华医学会杂志社远程稿件管理系统”查询稿件的审理结果。作者如需要采用通知或退稿通知可与编辑部联系。编辑部发给作者修改再版的稿件,如2个月没有修回,视为作者自行撤稿。编辑部的各种通知将通过Email发出,投稿后和稿件审理期间请作者留意自己的电子信箱。作者自收到采用通知之日起,即视为双方建立合约关系,作者如撤稿必须向编辑部申诉理由并征得编辑部同意。一旦稿件进入编排阶段,作者不应提出自撤稿件,在此期间因一稿两投或强行撤稿而给本刊造成不良影响和/或经济损失者,编辑部有权给以公开曝光、通报并实施经济赔偿,作者自行承担一切责任和后果。

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(本刊编辑部)