

# 复方野菊花眼贴对蓝光诱导小鼠睑板腺功能异常的防治作用及其机制

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**【摘要】** 目的 探讨复方野菊花眼贴对蓝光诱导小鼠睑板腺功能异常的防治作用及其机制。方法 将 64 只 15 周龄雄性 C57BL/6J 小鼠按照随机数字表法分成 2 个部分, 每部分 32 只, 分别进行预防实验和治疗实验。预防实验和治疗实验各 32 只小鼠分别按照随机数字随机分为正常组、蓝光组、溶剂组和眼贴组, 每组 8 只。预防实验中, 除正常组外, 各组小鼠采用波长 460 nm、光照度 2 000 lx 的蓝光, 每天暴露 6 h, 连续暴露 15 d 建立小鼠睑板腺功能异常模型; 溶剂组和眼贴组每天在蓝光暴露前和暴露后, 分别用相对应眼贴敷贴预防 25 min, 连续 15 d, 蓝光组仅接受蓝光暴露 15 d 处理, 并于第 15 天行睑板腺开口照相观察小鼠睑板腺功能。治疗实验中, 除正常组外, 各组小鼠均采用上述方法建立小鼠睑板腺功能异常模型, 溶剂组和眼贴组在结束 15 d 的蓝光暴露后, 每天早上和下午分别用相对应眼贴敷贴治疗 25 min, 连续 15 d, 蓝光组置于标准环境中观察 15 d, 并在第 15 天行睑板腺开口照相观察小鼠睑板腺功能变化。预防实验及治疗实验的各组小鼠在处理完成后进行离体睑板腺拍照、油红 O 染色、苏木精-伊红染色观察小鼠睑板腺组织学变化; 采用实时荧光定量 PCR 法检测各组睑板腺组织中炎性因子白细胞介素 1 $\beta$  (IL-1 $\beta$ )、IL-6、肿瘤坏死因子  $\alpha$  (TNF- $\alpha$ )、 $\gamma$  干扰素 (IFN- $\gamma$ ) mRNA 相对表达量; 采用 Western blot 法检测睑板腺组织中核因子 (NF)- $\kappa$ B 和磷酸化 NF- $\kappa$ B (p-NF- $\kappa$ B) 蛋白的表达, 评估复方野菊花眼贴对蓝光诱导小鼠睑板腺炎症的改善程度。结果 与正常组相比, 蓝光组暴露后 15 d 小鼠睑板腺开口阻塞数量逐渐增加, 睑板腺下睑相对剩余面积逐渐减小, 差异均有统计学意义 (均  $P < 0.05$ )。预防实验中, 眼贴组睑板腺开口阻塞数量为  $1.833 \pm 0.753$ , 明显少于溶剂组的  $3.667 \pm 1.033$ , 眼贴组睑板腺下睑相对剩余面积为  $0.718 \pm 0.091$ , 明显大于溶剂组的  $0.624 \pm 0.130$ , 差异均有统计学意义 (均  $P < 0.05$ )。苏木精-伊红染色结果显示, 蓝光组和溶剂组睑板腺有炎性细胞浸润; 眼贴组无炎性细胞浸润, 腺泡形态接近正常组; 油红 O 染色结果显示各组睑板腺均无明显脂质沉积。眼贴组 IL-1 $\beta$ 、IL-6、TNF- $\alpha$ 、IFN- $\gamma$  mRNA 相对表达量明显低于溶剂组, 眼贴组 NF- $\kappa$ B、p-NF- $\kappa$ B 蛋白相对表达量明显低于溶剂组, 差异均有统计学意义 (均  $P < 0.05$ )。治疗实验中, 眼贴组和溶剂组的睑板腺开口阻塞数量分别为  $4.333 \pm 1.211$  和  $4.833 \pm 1.722$ , 睑板腺下睑相对剩余面积分别为  $0.572 \pm 0.151$  和  $0.588 \pm 0.154$ , 2 个组比较差异均无统计学意义 (均  $P > 0.05$ )。苏木精-伊红染色结果显示, 蓝光组和溶剂组睑板腺有炎性细胞浸润, 眼贴组无炎性细胞浸润, 腺泡形态接近正常组; 油红 O 染色结果显示各组睑板腺均无明显脂质沉积。眼贴组 IL-1 $\beta$ 、IL-6、IFN- $\gamma$  mRNA 相对表达量明显低于溶剂组, 眼贴组 NF- $\kappa$ B、p-NF- $\kappa$ B 蛋白相对表达量明显低于溶剂组, 差异均有统计学意义 (均  $P < 0.05$ )。结论 复方野菊花眼贴可通过抑制 NF- $\kappa$ B 信号通路减轻睑板腺组织炎症反应, 对蓝光诱导的睑板腺功能异常具有防治作用。

**【关键词】** 炎症; 蓝光; 睑板腺功能异常; 复方野菊花眼贴

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## Preventive and therapeutic effects of compound wild chrysanthemum eye pad on blue light-induced alteration of meibomian gland function in mice and its mechanism

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**[Abstract] Objective** To investigate the preventive and therapeutic effects of compound wild chrysanthemum eye pad on blue light-induced alteration of meibomian gland function in mice and its mechanism.

**Methods** Sixty-four 15-week-old male C57BL/6J mice were divided into two groups of 32 mice each according to random numbers for the prevention test and the treatment test. The respective 32 mice in the prevention and treatment experiments were randomly divided into normal group, blue light group, solvent group and eye pad group according to random numbers, with eight mice in each group, respectively. In the prevention experiments, mice in each group were exposed to blue light at a wavelength of 460 nm and a light intensity of 2 000 lx for 6 hours per day for 15 consecutive days to establish a mouse model of meibomian gland function changes except for the normal group. The solvent group and the eye pad group were treated with the corresponding eye pad before and after the blue light exposure for 25 minutes daily for the 15 consecutive days. The blue light group was treated with blue light exposure only for 15 days, and the mice were photographed at the edge of the meibomian gland on day 15 to observe the function of the meibomian gland except for the normal group. In the treatment test, all groups of mice except the normal group were induced the altered function of the mouse meibomian gland by the above method. The solvent and eye pad groups were treated with corresponding eye pads for 25 minutes in the morning and afternoon of each day for 15 consecutive days after blue light exposure. The blue light group was kept in a standard environment for 15 days and the changes in meibomian gland function of mice were detected by meibomian gland photographs on day 15. Photography of the eyelid margin *in vitro*, oil red O staining, and hematoxylin-eosin staining were performed to observe the histologic changes in the meibomian glands of mice after the preventive and experimental treatment. The relative expression of interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interferon- $\gamma$  (IFN- $\gamma$ ) mRNA in mouse meibomian gland tissues was detected by real-time fluorescence quantitative PCR. The expression of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and phosphorylation of NF- $\kappa$ B (p-NF- $\kappa$ B) proteins in mice meibomian gland tissues was detected by Western blot to assess the degree of amelioration of blue light-induced inflammation in mouse meibomian glands by the compound wild chrysanthemum eye pad. This study was conducted in accordance with the Statement of the Association for Research in Vision and Ophthalmology on the Use of Animals in Ophthalmology and Vision Research, and was approved by the Animal Ethics Committee of Xiamen University (No. XMULAC20220258).

**Results** Compared with the normal group, a gradually increased number of blocked meibomian gland openings, and a gradually decreased remaining area of lower meibomian gland, were observed in the mice after 15 days of blue light group, and all the differences were statistically different (all at  $P < 0.05$ ). In the prevention test, the number of obstructed opening in the eye pad group was  $1.833 \pm 0.753$ , which was significantly less than  $3.667 \pm 1.033$  in the solvent group ( $P < 0.05$ ). The relative remaining area of the lower lid meibomian gland in the eye pad group was  $0.718 \pm 0.091$ , which was significantly greater than  $0.624 \pm 0.130$  in the solvent group ( $P < 0.05$ ). Hematoxylin-eosin staining showed inflammatory cell infiltration in mouse meibomian gland in the blue light and solvent groups. There was no inflammatory cell infiltration in eye pad group, and the morphology of the acini was similar to that of the normal group. Oil red O staining showed that there was no significant lipid deposition in the groups. The relative expressions of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IFN- $\gamma$  mRNA were significantly lower, and the relative expressions of NF- $\kappa$ B and p-NF- $\kappa$ B proteins were significantly lower in the eye pad group than in the solvent group, showing statistically significant differences (all at  $P < 0.05$ ). In the treatment test, the number of obstructed openings in the eye pad group and solvent group was  $4.333 \pm 1.211$  and  $4.833 \pm 1.722$ , respectively, and the relative remaining area of the lower meibomian gland was  $0.572 \pm 0.151$  and  $0.588 \pm 0.154$ , respectively, showing no statistically significant differences (both at  $P > 0.05$ ). Hematoxylin-eosin staining showed inflammatory cell infiltration in mouse meibomian glands in the blue light and solvent groups, with a similar morphology of acini as in the normal group. There was no inflammatory cell infiltration in eye pad group. Oil red O staining showed that there was no significant lipid deposition in the groups. The relative expressions of IL-1 $\beta$ , IL-6, and IFN- $\gamma$  mRNA were significantly lower and the relative expressions of NF- $\kappa$ B and p-NF- $\kappa$ B proteins were significantly lower in the eye pad group than in the solvent group (all at  $P < 0.05$ ).

**Conclusions** Compound wild chrysanthemum eye pad may have preventive and therapeutic effects on blue light-induced changes in meibomian gland function by reducing the inflammatory response of meibomian gland tissue through the inhibition of the NF- $\kappa$ B signaling pathway.

**[Key words]** Inflammation; Blue light; Meibomian gland dysfunction; Compound wild chrysanthemum eye pad

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睑板腺是位于上眼睑和下眼睑的一种特殊皮脂腺,在睑板中呈上下垂直排列<sup>[1]</sup>。睑板腺通过分泌特定的脂质来维持眼表泪膜的稳态,脂质的主要功能是润滑眼表以防止眼表水分挥发,并保护眼表免受外来病原体的威胁<sup>[2-4]</sup>。睑板腺功能障碍(meibomian gland dysfunction, MGD)以慢性炎症反应为中心环节,是一种以睑板腺终末导管萎缩和/或睑酯分泌的量或质发生异常为主要特征的慢性、弥漫性睑板腺病变,可导致泪膜稳定性改变,产生眼表炎症和刺激症状<sup>[5]</sup>,是导致干眼的主要原因。MGD 的全球患病率为 3.5%~70.0%<sup>[6]</sup>,目前其治疗方式主要分为物理治疗、药物治疗和手术<sup>[7]</sup>,但既往方法普遍存在治疗成本高、操作难度较大、治疗周期长、效果较差、对患者伤害较大、不能完全满足患者的需求等问题。有研究表明,复方野菊花眼贴在干眼治疗中获得了较好的临床效果<sup>[8]</sup>,其是否对干眼主要致病因素之一的 MGD 也具有防治作用尚不清楚。本研究拟观察复方野菊花眼贴对蓝光引起的睑板腺功能异常的防治作用,探讨复方野菊花眼贴改善睑板腺功能异常的效果及可能的机制,为未来开发预防和治疗 MGD 相关药物提供理论支持。

## 1 材料与方法

### 1.1 材料

**1.1.1 实验动物及分组** 15 周龄成年雄性 SPF 级 C57BL/6J 小鼠 64 只,购自上海 SLAC 实验动物中心[许可证号:SYXK(闽):2018-0009]。本研究严格遵循视觉与眼科研究协会(ARVO)关于动物用于眼科和视觉研究的声明,并经厦门大学动物伦理委员会批准(批文号:XMULAC20220258)。小鼠可自由饮食和饮水,在(25±1)℃、相对湿度(60±10)%和交替 12 h 明暗循环(8:00~20:00)的标准无病原体环境中饲养。预防实验和治疗实验中各 32 只小鼠分别按随机数字表法随机分为正常组、蓝光组、溶剂组和眼贴组,每组 8 只。

**1.1.2 主要试剂及仪器** 兔抗小鼠核因子(nuclear factor, NF)- $\kappa$ B 抗体(4764S)、兔抗小鼠磷酸化 NF- $\kappa$ B(phosphorylation of NF- $\kappa$ B, p-NF- $\kappa$ B)抗体(3039S)(美国 Cell Signaling Technology 公司);山羊抗兔二抗(ab6721)、兔抗小鼠  $\beta$ -actin 抗体(ab227387)(美国 Abcam 公司);苏木精-伊红染色试剂盒(C0105S-2)(上海碧云天生物技术有限公司);改良油红 O 染色液(BL987A)(安徽 Biosharp 公司)。SLM-7E 型裂隙灯显微镜(山东康华科技有限公司);CX23 光学显微镜(日本 Olympus 公司);M165FC 正置荧光显微镜、CM1850 冰冻切片仪、EG1160 石蜡切片仪(德国 Leica

公司);ZK-10-VISU-150 手术显微镜(德国蔡司公司)。

### 1.2 方法

**1.2.1 眼贴的制备** 通过全封闭动态回流多功能提取罐控温萃取甄选的多味中药材,确保提取的无污染、高浓度和充分性;过滤、静置沉淀后将上清液经 60~70℃真空浓缩、16 300 r/min 离心 30 min 并用超速离心管分离上清,形成提取浓缩液;将提取浓缩液置于冷库中静置沉淀,制剂后进行灌装,最终制成眼贴。

#### 1.2.2 实验动物的分组处理

**1.2.2.1 睑板腺功能异常动物模型** 将小鼠按照随机数字表法分成正常组 32 只和蓝光组 32 只。正常组和蓝光组各 32 只小鼠再分别按照随机数字表法随机分为 0 d 组、7 d 组、15 d 组和 30 d 组,每组 8 只。正常组和蓝光组均置于标准无病原体环境中饲养,蓝光组小鼠采用波长 460 nm、光照度 2 000 lx 的蓝光,每天暴露 6 h,分别在蓝光暴露 0、7、15 和 30 d 时进行睑板腺开口拍照、离体睑板腺拍照,观察小鼠睑板腺组织学变化。

**1.2.2.2 预防实验部分** 正常组小鼠置于标准环境下饲养,其余各组在标准环境的基础上进行蓝光暴露(波长为 460 nm,光照度为 2 000 lx),每天 6 h(8:00~14:00),连续暴露 15 d 建立小鼠睑板腺功能异常模型;溶剂组和眼贴组在蓝光暴露的 15 d 中每天进行 2 次相对应的眼贴敷贴预防治疗(7:00、15:00),每次 25 min。在第 15 天对各组小鼠行睑板腺开口拍照,观察小鼠睑板腺功能情况,并采用颈椎脱臼法处死小鼠,取小鼠睑板腺进行睑板腺拍照、油红 O 染色、苏木精-伊红染色、实时荧光定量 PCR 和 Western blot 实验。

**1.2.2.3 治疗实验部分** 正常组小鼠置于标准环境下饲养,其余各组在标准环境的基础上进行蓝光暴露(波长为 460 nm,光照度为 2 000 lx),每天 6 h(8:00~14:00),持续 15 d。蓝光组在蓝光暴露结束后不作任何处理,溶剂组和眼贴组在蓝光暴露结束后,每天进行 2 次相对应的双眼眼贴敷贴治疗(7:00、15:00),每次 25 min,持续 15 d。在第 15 天对各组行睑板腺开口拍照,观察小鼠眼表及睑板腺功能,并采用颈椎脱臼法处死小鼠,取小鼠睑板腺进行睑板腺拍照、油红 O 染色、苏木精-伊红染色、实时荧光定量 PCR 和 Western blot 实验。

#### 1.2.3 小鼠睑板腺形态检查

**1.2.3.1 睑板腺开口拍照** 操作者用右手将小鼠固定好,左手调整裂隙灯显微镜,保持灯光与小鼠垂直,尽可能保证小鼠睑缘不反光;另一操作者将裂隙灯显微镜焦距调好同时确定拍摄倍数,至屏幕显示出清晰图像即可。

**1.2.3.2 离体睑板腺拍照** 采用颈椎脱臼法处死各



组小鼠,迅速取下小鼠上下眼睑,去除多余组织后充分暴露小鼠睑板腺,置于正置显微下观察并拍照。采用 ImageJ 软件(美国 National Institute of Health 公司)计算睑板腺剩余面积。睑板腺相对剩余面积=睑板腺剩余面积/睑板腺面积。

#### 1.2.4 小鼠睑板腺组织病理学检查

**1.2.4.1 苏木精-伊红染色观察小鼠睑板腺结构和炎性细胞浸润情况** 各组任意选取 3 块睑板腺组织,用 2 块载玻片将组织轻轻压平并摊开,用 4% 多聚甲醛固定组织 30 min,待睑板腺初步固定后,将睑板腺轻轻转移至装有 1 ml 4% 多聚甲醛的 EP 管中固定 24 h,用双蒸水冲洗掉多余多聚甲醛,常温下梯度乙醇脱水(70%、80%、95%、100%、100%、100% 乙醇各 2 min),后用二甲苯进一步脱水(二甲苯 I、II、III 各 2 min)以及二甲苯透明后浸蜡包埋。采用 Leica EG1160 石蜡切片机切片,切片方向为垂直于睑板腺腺体方向,切片厚度为 5  $\mu\text{m}$ ,每个组织制备 5 个睑板腺组织切片,苏木精-伊红染色,光学显微镜下选取睑板腺中央 5 个腺体组织,观察睑板腺腺体结构改变以及腺体间细胞增生情况并拍照。采用 ImageJ 软件进行组织间炎性细胞计数。

**1.2.4.2 油红 O 染色观察各组小鼠睑板腺脂质沉积情况** 各组任意选取 3 块睑板腺组织,用专用吸水纸吸干多余水分,同时用 2 把显微镊轻轻夹住组织边缘并将睑板腺尽可能展开,另一人再次用吸水纸吸干多余水分,再平整地将其置于装有 OCT 溶液的包埋盒中,待睑板腺组织中气泡完全排出后,用镊子夹取包埋盒缓慢放入液氮中固定。使用冰冻切片机于平行睑板腺腺体方向行 5  $\mu\text{m}$  厚切片,每块组织制备 5 个睑板腺组织切片。进行油红 O 染色,光学显微镜下选取睑板腺中央 5 块腺体组织观察睑板腺腺体中脂质堆积情况并拍照。采用 ImageJ 软件统计睑板腺组织中脂质沉积情况。

**1.2.5 实时荧光定量 PCR 法检测各组睑板腺组织中白细胞介素 1 $\beta$ 、白细胞介素 6、肿瘤坏死因子  $\alpha$ 、 $\gamma$  干扰素 mRNA 表达** 实验结束后将小鼠全部安乐死,采用随机数字表法随机选取 6 只小鼠,将小鼠睑板腺取出后置于含 1 ml Trizol 的 EP 管中,采用 Trizol 法提取组织 RNA,按照说明书逆转录成 cDNA。引物由上海生工生物工程有限公司合成,其引物名称及序列分别为  $\beta$ -actin 正向引物:5'-AGATCAAGATCATTGCTCC TCCT-3',反向引物:5'-ACGCAGCTCAGTAACAGTCC-3';白细胞介素 1 $\beta$ (interleukin-1 $\beta$ , IL-1 $\beta$ )正向引物:5'-GCACTACAGGCTCCGAGATGAA-3',反向引物:5'-

GTCGTTGCTTGGTTCTCCTTGT-3';IL-6 正向引物:5'-CTTGGGACTGATGCTGGTGACA-3',反向引物:5'-GCCTCCGACTTGTGAAGTGGA-3';肿瘤坏死因子  $\alpha$ (tumor necrosis factor- $\alpha$ , TNF- $\alpha$ )正向引物:5'-ACAGCAAGGGACTAGCCAGGAG-3',反向引物:5'-AGTGCCTCTTCTGCCAGTTCCA-3'; $\gamma$  干扰素(interferon- $\gamma$ , INF- $\gamma$ )正向引物:5'-CTTCAGCAACAGCAAGGCGAAA-3',反向引物:5'-CCGAATCAGCAGCGACTCCT-3'。进行实时荧光定量 PCR 反应,PCR 反应条件:95  $^{\circ}\text{C}$  预变性 10 min;95  $^{\circ}\text{C}$  变性 10 s,60  $^{\circ}\text{C}$  退火和延伸 30 s,进行 40 个循环。以  $\beta$ -actin 为内参,采用  $2^{-\Delta\Delta\text{Ct}}$  法计算各目的基因相对表达量。

**1.2.6 Western blot 法检测睑板腺组织中 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白表达** 采用随机数字表法随机选取 3 只小鼠左眼睑板腺,置于含有 0.1 ml RIPA 的 EP 管中,研磨组织收集蛋白,进行 SDS-PAGE 电泳并转膜至醋酸纤维素膜中,2% BSA 封闭 1 h;分别加入 NF- $\kappa$ B 一抗(1:1 000)、p-NF- $\kappa$ B 一抗(1:1 000)4  $^{\circ}\text{C}$  孵育过夜,1 倍 TBST 漂洗 3 次,每次 5~10 min,加入羊抗兔 IgG 二抗(1:500)孵育 1 h,1 倍 TBST 漂洗 3 次,每次 5~10 min,使用 Western Bright<sup>TM</sup> ECL 和 Western Bright<sup>TM</sup> Peroxide 按照 1:1 配制显影液,使用 BIO-RAD 显影仪显影。以  $\beta$ -actin 为内参,采用 ImageJ 软件分别计算内参和目的蛋白条带灰度值。目的蛋白相对表达量=目的蛋白条带灰度值/内参条带灰度值。

#### 1.3 统计学方法

采用 GraphPad Prism 8 软件进行统计分析。计量资料数据经 Shapiro-Wilk 检验证实呈正态分布,以  $\bar{x} \pm s$  表示。正常组与蓝光组间不同时间点睑板腺形态指标总体差异比较采用区组设计两因素方差分析,多个组间评估指标的总差异比较采用单因素方差分析,多重比较采用 LSD- $t$  检验。 $P < 0.05$  为差异有统计学意义。

## 2 结果

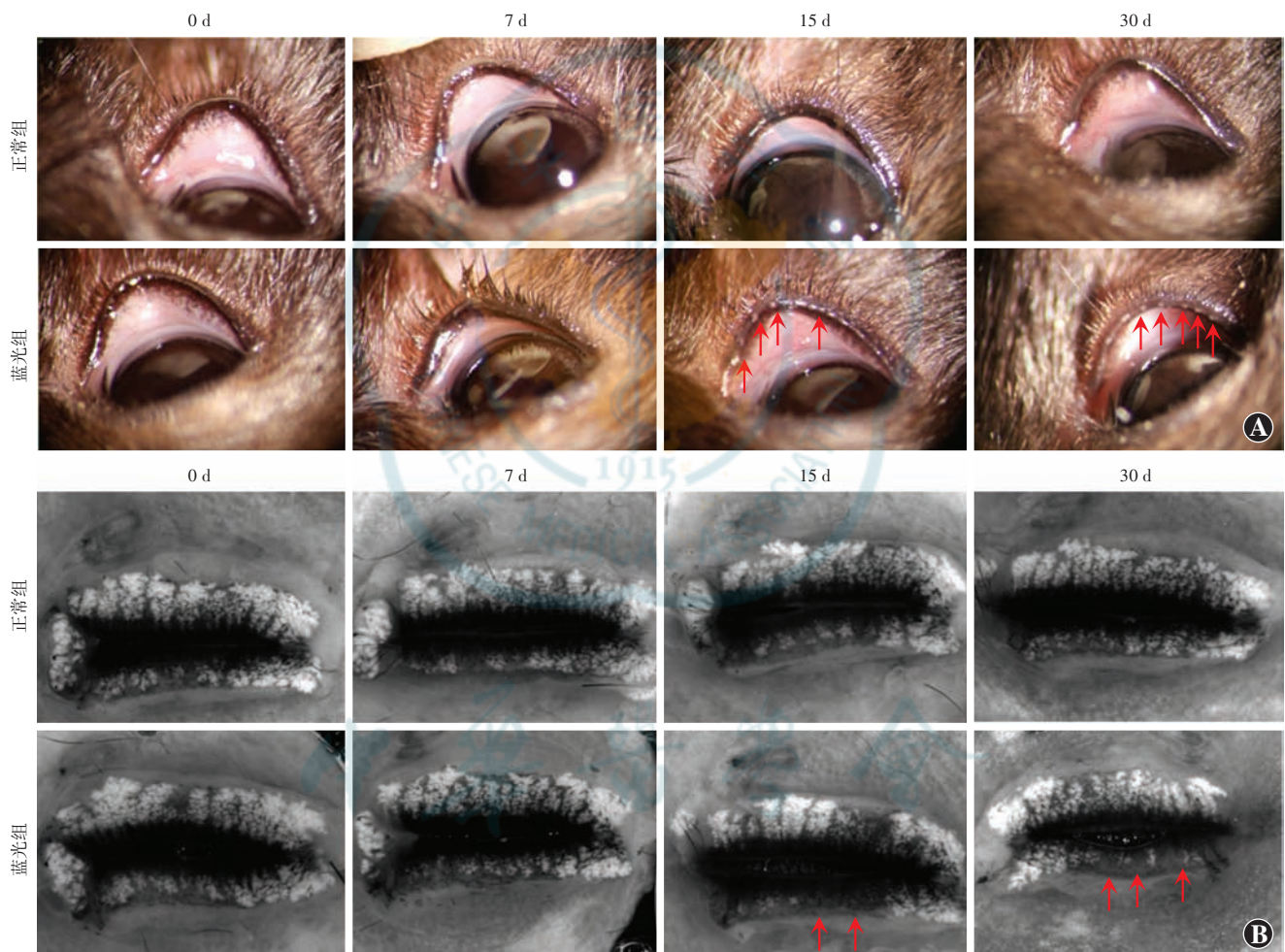
### 2.1 正常组与蓝光组小鼠眼睑板腺形态变化

**2.1.1 正常组与蓝光组小鼠睑板腺开口阻塞数量比较** 裂隙灯显微镜拍照显示,蓝光暴露前(0 d),正常组和蓝光组小鼠睑缘规整、光滑、无阻塞现象;7 d 时,正常组和蓝光组小鼠睑缘规整、光滑,少见睑板腺开口阻塞;15 d 时,蓝光组小鼠出现睑板腺开口阻塞和睑缘光滑程度下降,正常组睑缘无明显变化;30 d 时,蓝光组小鼠睑板腺开口明显阻塞,睑缘光滑程度明显下降,正常组睑缘无明显改变(图 1A)。正常组和蓝光组不同时间点小鼠睑板腺开口阻塞数量总体比较差异

均有统计学意义 ( $F_{\text{分组}} = 75.92, P < 0.001; F_{\text{时间}} = 40.32, P < 0.001$ ), 其中蓝光暴露后 15、30 d 蓝光组睑板腺开口阻塞数量明显多于正常组, 差异均有统计学意义 (均  $P < 0.05$ ) (表 1)。由于本实验重点关注早期睑板腺功能异常, 与暴露 15 d 相比, 蓝光暴露 30 d 睑板腺功能损伤过重, 故选用蓝光暴露 15 d 所诱导的睑板腺功能异常进行后续研究。

**2.1.2 正常组与蓝光组小鼠睑板腺下睑剩余面积比较** 离体睑板腺拍照显示, 蓝光暴露前 (0 d), 正常组和蓝光组小鼠睑板腺完整, 排列整齐; 7 d 时, 蓝光组

和正常组小鼠睑板腺基本完整, 未见明显缺失; 15 d 时, 蓝光组小鼠睑板腺形态出现异常并伴下睑腺体部分缺失, 正常组睑板腺结构完整; 30 d 时, 蓝光组小鼠睑板腺损伤明显, 下睑腺体基本缺失, 正常组睑板腺腺体形态完整 (图 1B)。正常组和蓝光组不同时间点小鼠睑板腺下睑相对剩余面积总体比较差异均有统计学意义 ( $F_{\text{分组}} = 258.30, P < 0.001; F_{\text{时间}} = 57.47, P < 0.001$ ), 其中蓝光暴露后 15、30 d 蓝光组小鼠睑板腺下睑相对剩余面积明显小于正常组, 差异均有统计学意义 (均  $P < 0.05$ ) (表 2)。



**图 1 正常组与蓝光组不同时间点小鼠睑缘及睑板腺形态变化比较** A: 裂隙灯显微镜下拍照 0 d 时, 正常组和蓝光组睑板腺开口通畅, 睑缘光滑; 7 d 时, 蓝光组较正常组睑缘光滑程度降低, 但未见明显睑板腺开口阻塞; 15 d 时, 蓝光组较正常组睑板腺开口出现阻塞, 睑缘光滑程度降低 (箭头); 30 d 时, 蓝光组较正常组睑板腺开口阻塞程度进一步加重, 睑缘不规整 (箭头) B: 离体睑板腺拍照 0 d 时, 正常组和蓝光组小鼠睑板腺完整, 排列整齐; 7 d 时, 蓝光组较正常组睑板腺基本完整, 未见明显缺失; 15 d 时, 蓝光组较正常组睑板腺形态出现异常, 下睑腺体部分缺失 (箭头); 30 d 时, 蓝光组较正常组睑板腺损伤明显, 下睑腺体基本缺失 (箭头)

**Figure 1 Comparison of morphological changes in mice eyelid margins and meibomian glands at different time points between normal group and blue light group** A: Slit lamp microscopy images On day 0, the mice meibomian gland opening was normal and the eyelid margin was smooth in the two groups. On the 7th day, the eyelid margin was less smooth in the blue light group compared to normal group, but no significant obstruction of the opening was seen. On the 15th day, the openings were blocked in the blue light group compared to the normal group, with a decrease in the smoothness of the eyelid margin (arrows). On the 30th day, obstruction of the opening was severer in the blue light group compared to the normal group, and the eyelid margins were irregular (arrows) B: Photographs of meibomian gland *in vitro* On day 0, the mice meibomian gland was intact and neatly arranged in the two groups. On the 7th day, the mice meibomian gland in the blue light group was generally intact without significant loss compared to normal group. On the 15th day, the mice meibomian gland in the blue light group was abnormal, showing partial absence of the lower meibomian gland (arrows) compared with normal group. On the 30th day, the mice meibomian gland damage was more obvious with whole absence of lower meibomian glands (arrows) in the blue light group than in the normal group



**表 1 各组小鼠蓝光暴露后不同时间点睑板腺开口阻塞数量比较 ( $\bar{x}\pm s$ , 个)**  
**Table 1 Comparison of the number of obstructed mice meibomian gland openings at different time points after blue light exposure between two groups ( $\bar{x}\pm s$ , pcs)**

组别	样本量	不同时间点睑板腺开口阻塞数量			
		0 d	7 d	15 d	30 d
正常组	8	0.000±0.000	0.250±0.433	0.625±0.484	1.000±0.707
蓝光组	8	0.125±0.331	0.875±0.781	3.625±0.857 <sup>a</sup>	6.125±1.452 <sup>a</sup>

注:  $F_{\text{分组}} = 75.92, P < 0.001; F_{\text{时间}} = 40.32, P < 0.001$ . 与各自时间点正常组比较, <sup>a</sup> $P < 0.05$  (两因素方差分析, LSD-*t* 检验)

Note:  $F_{\text{group}} = 75.92, P < 0.001; F_{\text{time}} = 40.32, P < 0.001$ . Compared with respective normal group, <sup>a</sup> $P < 0.05$  (Two-way ANOVA, LSD-*t* test)

**表 2 各组小鼠蓝光暴露后不同时间点睑板腺下睑相对剩余面积比较 ( $\bar{x}\pm s$ )**  
**Table 2 Comparison of the relative remaining area of lower mice meibomian at different time points after blue light exposure between two groups ( $\bar{x}\pm s$ )**

组别	样本量	不同时间点睑板腺下睑相对剩余面积			
		0 d	7 d	15 d	30 d
正常组	8	0.971±0.028	0.984±0.039	0.914±0.058	0.907±0.043
蓝光组	8	0.995±0.046	0.819±0.058	0.620±0.065 <sup>a</sup>	0.407±0.079 <sup>a</sup>

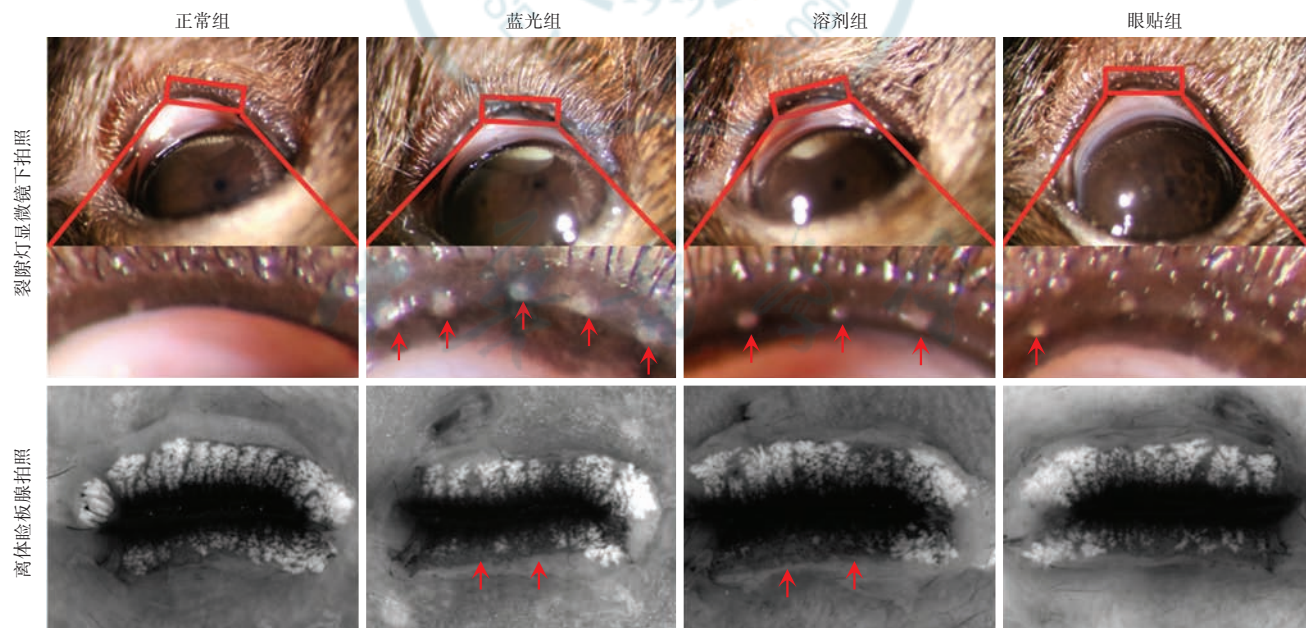
注:  $F_{\text{分组}} = 258.30, P < 0.001; F_{\text{时间}} = 57.47, P < 0.001$ . 与各自时间点正常组比较, <sup>a</sup> $P < 0.05$  (两因素方差分析, LSD-*t* 检验)

Note:  $F_{\text{group}} = 258.30, P < 0.001; F_{\text{time}} = 57.47, P < 0.001$ . Compared with respective normal group, <sup>a</sup> $P < 0.05$  (Two-way ANOVA, LSD-*t* test)

## 2.2 预防实验中各组小鼠睑板腺相关指标比较

裂隙灯显微镜照相显示, 蓝光照射 15 d 时, 正常组小鼠睑缘光滑, 无睑板腺开口阻塞; 蓝光组和溶剂组小鼠睑板腺开口阻塞明显, 睑缘增厚, 睑板腺受损; 眼贴组小鼠睑缘形态接近正常, 偶见睑板腺开口阻塞 (图 2)。各组睑板腺开口阻塞数量总体比较, 差异有统计学意义 ( $F = 20.12, P < 0.05$ ), 其中眼贴组睑板腺开口阻塞数量少于溶剂组, 差异有统计学意义 ( $P < 0.05$ ) (表 3)。

离体睑板腺拍照显示, 蓝光照射 15 d 时, 正常组小鼠睑板腺腺体结构完整; 蓝光组和溶剂组睑板腺受损, 腺体部分缺失; 眼贴组睑板腺腺体结构基本完整, 未观察到明显缺失 (图 2)。各组睑板腺下睑相对剩余面积总体比较, 差异有统计学意义 ( $F = 31.24, P < 0.05$ ), 其中眼贴组睑板腺下睑相对剩余面积明显大于溶剂组, 差异有统计学意义 ( $P < 0.05$ ) (表 4)。



**图 2 预防实验中 15 d 时各组小鼠睑板腺形态比较** 裂隙灯显微镜下拍照可见, 正常组小鼠睑板腺开口未见堵塞, 睑缘光滑; 蓝光组和溶剂组可见乳白色堵塞 (箭头), 睑缘不规则; 眼贴组偶见睑板腺开口阻塞 (箭头) (红框内为小鼠睑缘中央睑板腺开口所指)。离体睑板腺拍照可见, 正常组小鼠睑板腺排列整齐, 睑板腺完整; 蓝光组和溶剂组睑板腺形态不规则, 下睑板腺腺体缺失 (箭头); 眼贴组睑板腺腺体基本完整, 未见明显缺失

**Figure 2 Changes in mice meibomian gland tissue in different groups on day 15 in the prevention test** In slit lamp microscopy images, no significant obstruction of the openings was observed and the eyelid margin was smooth in normal group. In blue light and solvent groups, creamy obstructed openings (arrows) and irregular eyelid margins were seen. In eye pad group, few obstruction of the openings (arrows) were visible (Within the red frames were meibomian gland openings at the central mice eyelid margins). In photographs of meibomian gland *in vitro*, well-aligned and intact meibomian glands were seen. In blue light and solvent groups, irregular meibomian gland and absence of lower meibomian glands (arrows) were observed in normal group. In eye pad group, meibomian glands were intact and not missing

**表 3 预防实验各组小鼠睑板腺开口阻塞数量比较 ( $\bar{x}\pm s$ , 个)**  
**Table 3 Comparison of the number of blocked meibomian gland openings among different groups in the prevention test ( $\bar{x}\pm s$ , pcs)**

组别	样本量	睑板腺开口阻塞数量
正常组	6	0.500±0.837
蓝光组	6	3.667±1.633
溶剂组	6	3.667±1.033
眼贴组	6	1.833±0.753 <sup>a</sup>
F 值		20.12
P 值		<0.05

注:与溶剂组比较,<sup>a</sup> $P<0.05$ (单因素方差分析,LSD-*t* 检验)  
 Note: Compared with solvent group, <sup>a</sup> $P<0.05$  (One-way ANOVA, LSD-*t* test)

**表 4 预防实验各组小鼠睑板腺下睑相对剩余面积比较 ( $\bar{x}\pm s$ )**  
**Table 4 Comparison of the relative remaining area of lower mice meibomian gland among different groups in the prevention test ( $\bar{x}\pm s$ )**

组别	样本量	睑板腺下睑相对剩余面积
正常组	7	1.000±0.076
蓝光组	7	0.572±0.061
溶剂组	7	0.624±0.130
眼贴组	7	0.718±0.091 <sup>a</sup>
F 值		31.24
P 值		<0.05

注:与溶剂组比较,<sup>a</sup> $P<0.05$ (单因素方差分析,LSD-*t* 检验)  
 Note: Compared with solvent group, <sup>a</sup> $P<0.05$  (One-way ANOVA, LSD-*t* test)

**2.3 预防实验中各组小鼠睑板腺组织病理学表现**

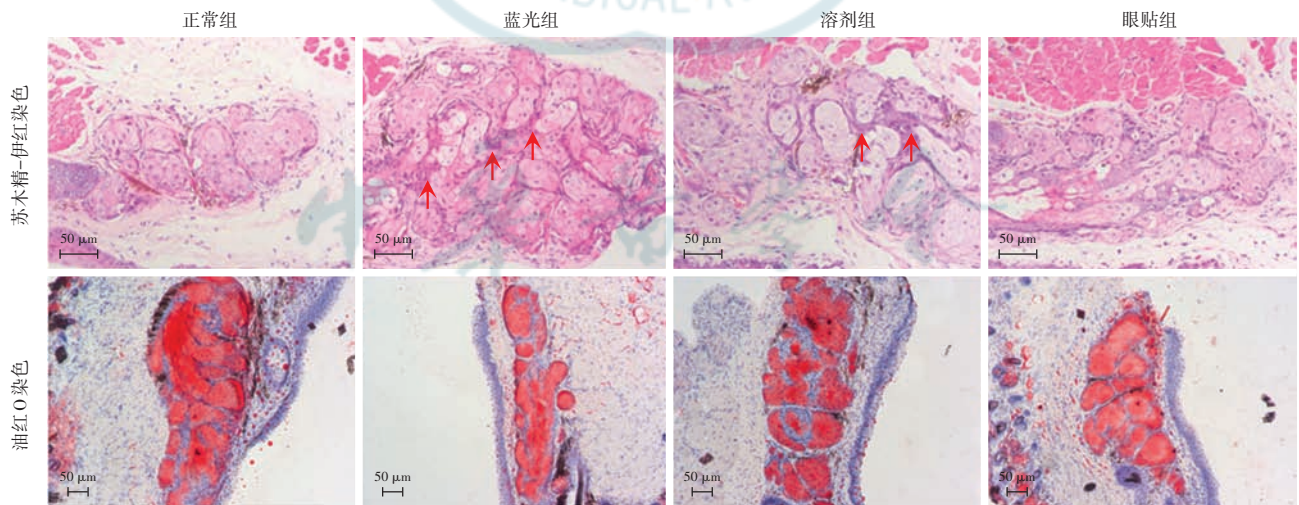
苏木精-伊红染色结果显示,正常组睑板腺腺体完整,结构清晰,细胞排列紧密,腺体间无炎性细胞浸润;蓝光组睑板腺腺体排列紊乱,腺体形态异常,呈现腺体萎缩的趋势,间充质细胞过度增加,腺体间出现炎性细胞浸润;溶剂组睑板腺腺体形态基本完整,睑板腺腺体间充质细胞过度增生,伴有炎性细胞浸润;眼贴组睑板腺腺体基本完整,形态规则,组织增生和炎性细胞浸润程度较溶剂组降低,呈现好转趋势。油红 O 染色结果显示,正常组、蓝光组、溶剂组、眼贴组睑板腺腺体、导管内无大量脂质沉积,形态结构正常(图 3)。

**2.4 预防实验中各组小鼠睑板腺组织中炎性因子相对表达量比较**

正常组、蓝光组、溶剂组和眼贴组 IL-1 $\beta$ 、IL-6、TNF- $\alpha$ 、IFN- $\gamma$  mRNA 相对表达量总体比较,差异均有统计学意义( $F = 50.56、26.97、66.81、23.71$ ,均  $P < 0.001$ ),其中眼贴组 IL-1 $\beta$ 、IL-6、TNF- $\alpha$ 、IFN- $\gamma$  mRNA 相对表达量较蓝光组和溶剂组明显降低,差异均有统计学意义(均  $P < 0.05$ )(表 5)。

**2.5 预防实验中各组小鼠睑板腺组织中 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白相对表达量比较**

Western blot 检测结果显示,蓝光组睑板腺组织中 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白灰度强于正常组,眼贴组 NF- $\kappa$ B



**图 3 预防实验中 15 d 时各组小鼠睑板腺组织病理学表现** 苏木精-伊红染色可见,正常组睑板腺腺体形态完整,细胞排列紧密,边界清晰,腺体间无组织增生和炎性细胞浸润;蓝光组腺体排列异常,边界不清,腺体间出现组织增生和炎性细胞浸润(箭头);溶剂组腺体形态基本正常,睑板腺腺体间组织增生并伴有炎性细胞浸润(箭头);眼贴组睑板腺腺体间组织增生减少,炎性细胞浸润程度降低( $\times 200$ ,标尺 = 50  $\mu$ m)。油红 O 染色可见,正常组、蓝光组、溶剂组和眼贴组睑板腺腺体及导管内均无明显脂质沉积( $\times 100$ ,标尺 = 50  $\mu$ m)

**Figure 3 Histopathological manifestations of mice meibomian gland in different groups on day 15 in the prevention test** By hematoxylin-eosin staining, intact meibomian gland in morphology with tightly arranged cells and clear borders were observed in normal group, and no tissue hyperplasia or inflammatory cell infiltration was seen between glands. In the blue light group, abnormally arranged meibomian glands with unclear borders, tissue hyperplasia and inflammatory cell infiltration between glands (arrows). In the solvent group, basically normal gland with inter-gland tissue hyperplasia and inflammatory cell infiltration (arrows) were seen. Reduced inter-gland tissue hyperplasia and inflammatory cell infiltration were seen in the eye pad group ( $\times 200$ , scale bar = 50  $\mu$ m). By oil red O staining, no significant lipid deposition was seen in the meibomian glands and ducts in the four groups ( $\times 100$ , scale bar = 50  $\mu$ m)



表 5 预防实验各组小鼠睑板腺组织中 IL-1β、IL-6、TNF-α、IFN-γ mRNA 相对表达量比较 ( $\bar{x} \pm s$ )

Table 5 Comparison of relative expressions of IL-1β, IL-6, TNF-α and IFN-γ mRNA in mice meibomian gland tissue among different groups in the prevention test ( $\bar{x} \pm s$ )

组别	样本量	IL-1β	IL-6	TNF-α	IFN-γ
正常组	6	1.006±0.050	1.000±0.136	1.009±0.064	1.004±0.094
蓝光组	6	6.453±0.918 <sup>a</sup>	3.681±0.921 <sup>a</sup>	11.067±1.631 <sup>a</sup>	3.972±0.852 <sup>a</sup>
溶剂组	6	6.101±0.754	4.713±0.806	10.613±1.232	3.489±0.840
眼贴组	6	3.776±1.044 <sup>bc</sup>	2.816±0.567 <sup>bc</sup>	7.314±1.481 <sup>bc</sup>	2.625±0.985 <sup>bc</sup>
F 值		50.56	26.97	66.81	23.71
P 值		<0.001	<0.001	<0.001	<0.001

注:与正常组比较,<sup>a</sup> $P < 0.05$ ;与蓝光组比较,<sup>b</sup> $P < 0.05$ ;与溶剂组比较,<sup>c</sup> $P < 0.05$ (单因素方差分析, LSD-*t* 检验) IL:白细胞介素;TNF:肿瘤坏死因子;IFN:干扰素

Note:Compared with normal group,<sup>a</sup> $P < 0.05$ ;compared with blue light group,<sup>b</sup> $P < 0.05$ ;compared with solvent group,<sup>c</sup> $P < 0.05$ (One-way ANOVA, LSD-*t* test) IL:interleukin;TNF:tumor necrosis factor;IFN:interferon

和 p-NF-κB 蛋白灰度弱于溶剂组(图 4)。正常组、蓝光组、溶剂组和眼贴组睑板腺组织中 NF-κB 和 p-NF-κB 蛋白相对表达量总体比较,差异均有统计学意义( $F = 37.85、57.64$ ,均  $P < 0.001$ ),其中眼贴组 NF-κB 和 p-NF-κB 蛋白相对表达量明显低于溶剂组,差异均有统计学意义(均  $P < 0.05$ )(表 6)。

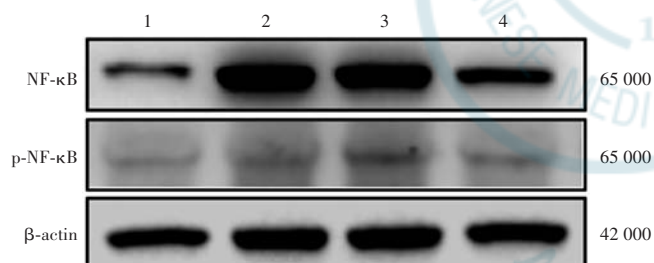


图 4 预防实验各组睑板腺组织中 NF-κB 和 p-NF-κB 蛋白表达电泳图 蓝光组 NF-κB 和 p-NF-κB 蛋白灰度强于正常组,眼贴组 NF-κB 和 p-NF-κB 蛋白灰度弱于溶剂组 1:正常组;2:蓝光组;3:溶剂组;4:眼贴组 NF-κB:核因子 κB;p-NF-κB:磷酸化核因子 κB;β-actin:β-肌动蛋白

Figure 4 Electrophoretogram of NF-κB and p-NF-κB protein expression in mice meibomian gland tissue in different groups in the prevention test Grayscale intensities of NF-κB and p-NF-κB proteins were stronger in the blue light group than in the normal group, weaker in the eye pad group than in the solvent group 1:normal group;2:blue light group;3:solvent group;4:eye pad group NF-κB:nuclear factor-κB;p-NF-κB:phosphorylation of nuclear factor-κB

## 2.6 治疗实验各组小鼠睑板腺相关指标比较

裂隙灯显微镜照相显示,蓝光照射 15 d 时,正常组小鼠睑缘光滑,无睑板腺开口阻塞;蓝光组、溶剂组和眼贴组小鼠睑板腺开口阻塞明显,睑缘增厚,睑板腺受损(图 5)。各组睑板腺开口阻塞数量总体比较,差异有统计学意义( $F = 28.34, P < 0.05$ ),其中蓝光组和

眼贴组睑板腺开口阻塞数量明显多于正常组,差异均有统计学意义(均  $P < 0.05$ )(表 7)。

离体睑板腺拍照显示,蓝光照射 15 d 时,正常组小鼠睑板腺腺体结构完整,蓝光组、溶剂组和眼贴组睑板腺受损,下睑腺体部分缺失(图 5)。各组睑板腺下睑相对剩余面积总体比较,差异有统计学意义( $F = 31.27, P < 0.05$ ),其中蓝光组和眼贴组睑板腺下睑相对剩余面积明显小于正常组,差异均有统计学意义(均  $P < 0.05$ )(表 8)。

表 6 预防实验各组小鼠睑板腺组织中 NF-κB 和 p-NF-κB 蛋白相对表达量比较 ( $\bar{x} \pm s$ )

Table 6 Comparison of the relative expression of NF-κB and p-NF-κB proteins in mice meibomian gland tissue among different groups in the prevention test ( $\bar{x} \pm s$ )

组别	样本量	NF-κB	p-NF-κB
正常组	3	1.000±0.348	1.000±0.202
蓝光组	3	2.361±0.549 <sup>a</sup>	1.066±0.850 <sup>a</sup>
溶剂组	3	1.977±0.146	1.150±0.114
眼贴组	3	1.631±0.233 <sup>bc</sup>	0.933±0.182 <sup>c</sup>
F 值		37.85	57.64
P 值		<0.001	<0.001

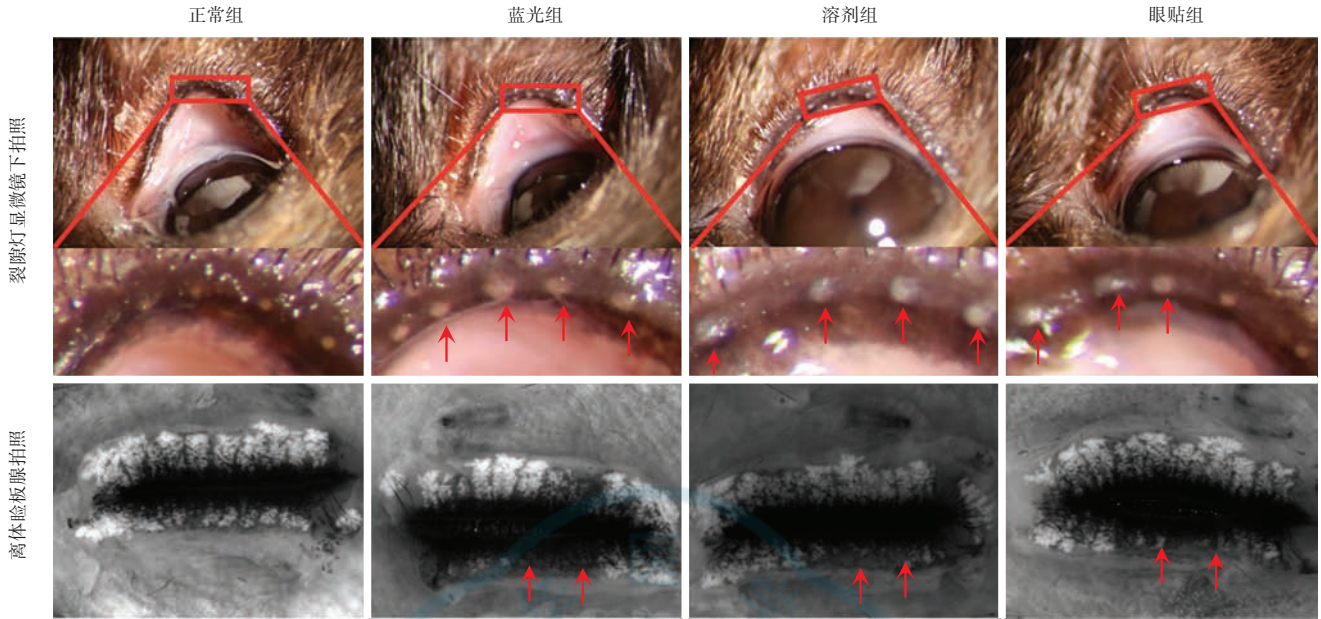
注:与正常组比较,<sup>a</sup> $P < 0.05$ ;与蓝光组比较,<sup>b</sup> $P < 0.05$ ;与溶剂组比较,<sup>c</sup> $P < 0.05$ (单因素方差分析, LSD-*t* 检验) NF-κB:核因子 κB;p-NF-κB:磷酸化核因子 κB

Note:Compared with normal group,<sup>a</sup> $P < 0.05$ ;compared with blue light group,<sup>b</sup> $P < 0.05$ ;compared with solvent group,<sup>c</sup> $P < 0.05$ (One-way ANOVA, LSD-*t* test) NF-κB:nuclear factor-κB;p-NF-κB:phosphorylation of nuclear factor-κB

## 2.7 治疗实验各组小鼠睑板腺组织病理学表现

苏木精-伊红染色结果显示,正常组睑板腺腺体完整,结构清晰,细胞排列紧密,腺体间无炎性细胞浸润;蓝光组睑板腺腺体排列紊乱,腺体形态异常,分界不清,间充质细胞过度增加,腺体间出现炎性细胞浸润;溶剂组睑板腺腺体形态基本完整,睑板腺腺体间充质细胞过度增生,伴有炎性细胞浸润;眼贴组睑板腺腺体基本完整,形态规则,组织增生和炎性细胞浸润较溶剂组减轻。油红 O 染色结果显示,正常组、蓝光组、溶剂组和眼贴组睑板腺腺体及导管内均无大量脂质沉积,形态结构正常(图 6)。





**图 5 治疗实验 15 d 时各组小鼠睑板腺组织变化比较** 裂隙灯显微镜下拍照可见,正常组小鼠睑板腺开口未见明显栓塞;蓝光组、溶剂组和眼贴组睑板腺可见乳白色栓塞(箭头),睑缘不规则(红框内为小鼠睑缘中央睑板腺开口所指)。离体睑板腺拍照可见,正常组睑板腺排列整齐,睑板腺完整;蓝光组、溶剂组和眼贴组睑板腺形态不规则,下睑板腺腺体缺失(箭头)

**Figure 5 Changes in mice meibomian gland tissue in different groups on day 15 in the treatment test** In slit lamp microscopy images, no significant obstruction of the openings was observed in normal group. In blue light group, solvent group, and eye pad group, creamy obstructed openings (arrows) and irregular eyelid margins were seen (Within the red frames were meibomian gland openings at the central mice eyelid margins). In photographs of meibomian gland *in vitro*, well-aligned and intact meibomian glands were seen in normal group. In the blue light group, solvent group, and eye pad group, irregular meibomian gland and absence of lower meibomian glands (arrows) were observed

**表 7 治疗实验各组小鼠睑板腺开口阻塞数量比较 ( $\bar{x} \pm s$ , 个)**  
**Table 7 Comparison of the number of obstructed mice meibomian gland openings among different groups in the treatment test ( $\bar{x} \pm s$ , pcs)**

组别	样本量	睑板腺开口阻塞数量
正常组	6	1.000 ± 0.894
蓝光组	6	4.500 ± 1.643 <sup>a</sup>
溶剂组	6	4.833 ± 1.722
眼贴组	6	4.333 ± 1.211 <sup>a</sup>
F 值		28.34
P 值		<0.05

注:与正常组比较,<sup>a</sup> $P < 0.05$ (单因素方差分析,LSD-*t* 检验)  
Note: Compared with normal group,<sup>a</sup> $P < 0.05$  (One-way ANOVA, LSD-*t* test)

**表 8 治疗实验各组小鼠睑板腺下睑相对剩余面积比较 ( $\bar{x} \pm s$ )**  
**Table 8 Comparison of the relative remaining area of lower mice meibomian gland among different groups in the treatment test ( $\bar{x} \pm s$ )**

组别	样本量	睑板腺下睑相对剩余面积
正常组	7	1.000 ± 0.103
蓝光组	7	0.542 ± 0.100 <sup>a</sup>
溶剂组	7	0.588 ± 0.154
眼贴组	7	0.572 ± 0.151 <sup>a</sup>
F 值		31.27
P 值		<0.05

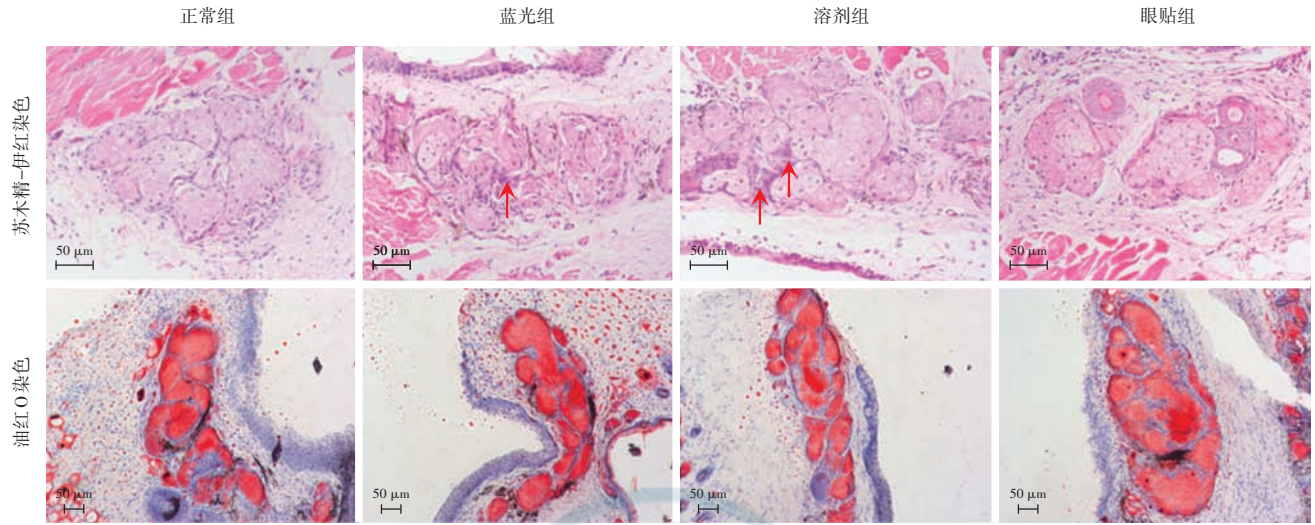
注:与正常组比较,<sup>a</sup> $P < 0.05$ (单因素方差分析,LSD-*t* 检验)  
Note: Compared with normal group,<sup>a</sup> $P < 0.05$  (One-way ANOVA, LSD-*t* test)

## 2.8 治疗实验各组小鼠睑板腺组织中炎性因子相对表达量比较

实时荧光定量 PCR 检测结果显示,正常组、蓝光组、溶剂组和眼贴组睑板腺组织中 IL-1 $\beta$ 、IL-6、TNF- $\alpha$ 、IFN- $\gamma$  mRNA 相对表达量总体比较,差异均有统计学意义 ( $F = 45.04, 35.68, 39.94, 30.13$ , 均  $P < 0.001$ ),其中眼贴组 IL-1 $\beta$ 、IL-6 和 IFN- $\gamma$  mRNA 相对表达量明显低于蓝光组和溶剂组,差异均有统计学意义 (均  $P < 0.05$ );眼贴组 TNF- $\alpha$  mRNA 相对表达量与蓝光组和溶剂组比较,差异均无统计学意义 (均  $P > 0.05$ ) (表 9)。

## 2.9 治疗实验各组小鼠睑板腺组织中 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白表达量比较

Western blot 检测结果显示,蓝光组睑板腺组织中 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白灰度强于正常组,眼贴组 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白灰度弱于溶剂组 (图 7)。正常组、蓝光组、溶剂组和眼贴组睑板腺组织中 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白相对表达量总体比较,差异均有统计学意义 ( $F = 43.13, P < 0.001; F = 21.31, P = 0.004$ ),其中眼贴组 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白相对表达量明显低于蓝光组和溶剂组,差异均有统计学意义 (均  $P < 0.05$ ) (表 10)。



**图 6 治疗实验 15 d 时各组小鼠睑板腺组织病理学表现** 苏木精-伊红染色结果显示,正常组睑板腺腺体形态完整,细胞排列紧密,边界清晰,腺体间无组织增生和炎性细胞浸润;蓝光组和溶剂组均表现为腺体排列紊乱(箭头),边界不清,腺体形态异常,腺体间组织增生明显并出现炎性细胞浸润;眼贴组睑板腺腺体结构基本完整,形态规则,腺体间组织增生减少,炎性细胞浸润程度较溶剂组减轻( $\times 200$ ,标尺=50  $\mu\text{m}$ )。油红 O 染色结果显示,正常组、蓝光组、溶剂组和眼贴组睑板腺腺体及导管内均无明显脂质沉积,形态结构正常( $\times 100$ ,标尺=50  $\mu\text{m}$ )

**Figure 6 Histopathological manifestations of mice meibomian gland in different groups on day 15 in the treatment test** By hematoxylin-eosin staining, intact meibomian gland in morphology with tightly arranged cells and clear borders were observed in normal group, and no tissue hyperplasia or inflammatory cell infiltration was seen between glands. In blue light and solvent groups, disordered meibomian glands (arrows) with unclear borders and abnormal morphology, obvious tissue hyperplasia and inflammatory cell infiltration between glands. Meibomian glands with intact structure, regular morphology, reduced inter-gland tissue hyperplasia and inflammatory cell infiltration than solvent group were seen in eye pad group ( $\times 200$ , scale bar = 50  $\mu\text{m}$ ). By oil red O staining, regular morphology and structure without significant lipid deposition was seen in the meibomian glands and ducts in four groups ( $\times 100$ , scale bar = 50  $\mu\text{m}$ )

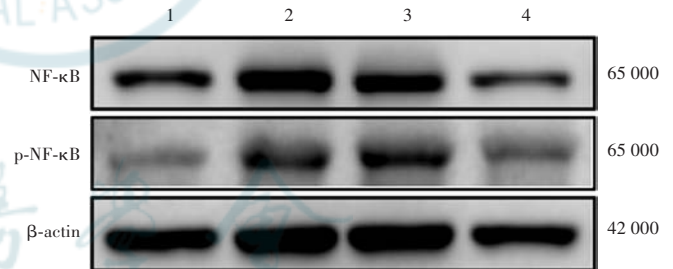
**表 9 治疗实验各组小鼠睑板腺组织中 IL-1 $\beta$ 、IL-6、TNF- $\alpha$ 、IFN- $\gamma$  mRNA 相对表达量比较 ( $\bar{x} \pm s$ )**

**Table 9 Comparison of relative expressions of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IFN- $\gamma$  mRNA in mice meibomian gland tissue among different groups in the treatment test ( $\bar{x} \pm s$ )**

组别	样本量	IL-1 $\beta$	IL-6	TNF- $\alpha$	IFN- $\gamma$
正常组	6	1.006 $\pm$ 0.164	0.999 $\pm$ 0.129	1.004 $\pm$ 0.120	0.994 $\pm$ 0.133
蓝光组	6	6.783 $\pm$ 1.232 <sup>a</sup>	6.487 $\pm$ 1.451 <sup>a</sup>	11.749 $\pm$ 2.255 <sup>a</sup>	7.292 $\pm$ 0.960 <sup>a</sup>
溶剂组	6	6.995 $\pm$ 0.770	6.684 $\pm$ 0.705	13.153 $\pm$ 2.673	7.154 $\pm$ 0.992
眼贴组	6	4.010 $\pm$ 1.161 <sup>bc</sup>	4.923 $\pm$ 1.131 <sup>bc</sup>	11.953 $\pm$ 1.938	4.887 $\pm$ 1.996 <sup>bc</sup>
F 值		45.04	35.68	39.94	30.13
P 值		<0.001	<0.001	<0.001	<0.001

注:与正常组比较,<sup>a</sup> $P < 0.05$ ;与蓝光组比较,<sup>b</sup> $P < 0.05$ ;与溶剂组比较,<sup>c</sup> $P < 0.05$ (单因素方差分析,LSD- $t$ 检验) IL:白细胞介素;TNF:肿瘤坏死因子;IFN:干扰素

Note: Compared with normal group, <sup>a</sup> $P < 0.05$ ; compared with blue light group, <sup>b</sup> $P < 0.05$ ; compared with solvent group, <sup>c</sup> $P < 0.05$  (One-way ANOVA, LSD- $t$  test) IL: interleukin; TNF: tumor necrosis factor; IFN: interferon



**图 7 治疗实验各组睑板腺组织中 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白表达电泳图** Western blot 检测结果显示,蓝光组 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白灰度强于正常组,眼贴组 NF- $\kappa$ B 和 p-NF- $\kappa$ B 蛋白灰度弱于溶剂组 1:正常组;2:蓝光组;3:溶剂组;4:眼贴组 NF- $\kappa$ B:核因子  $\kappa$ B;p-NF- $\kappa$ B:磷酸化核因子  $\kappa$ B; $\beta$ -actin: $\beta$ -肌动蛋白

**Figure 7 Electrophoretogram of NF- $\kappa$ B and p-NF- $\kappa$ B protein expressions in mice meibomian gland tissue in different groups in the treatment test** Western blot analysis showed that grayscale intensities of NF- $\kappa$ B and p-NF- $\kappa$ B proteins were stronger in blue light group than in normal group. Grayscale intensities of NF- $\kappa$ B and p-NF- $\kappa$ B proteins were weaker in eye pad group than in solvent group 1: normal group; 2: blue light group; 3: solvent group; 4: eye pad group NF- $\kappa$ B: nuclear factor- $\kappa$ B; p-NF- $\kappa$ B: phosphorylation of nuclear factor- $\kappa$ B



**表 10 治疗实验各组小鼠睑板腺组织中 NF-κB 和 p-NF-κB 蛋白相对表达量比较 ( $\bar{x} \pm s$ )**  
**Table 10 Comparison of the relative expression of NF-κB and p-NF-κB proteins in mice meibomian gland tissue among different groups in the treatment test ( $\bar{x} \pm s$ )**

组别	样本量	NF-κB	p-NF-κB
正常组	3	1.000±0.197	1.000±0.270
蓝光组	3	1.093±0.194 <sup>a</sup>	1.160±0.066 <sup>a</sup>
溶剂组	3	0.983±0.105	0.924±0.064
眼贴组	3	0.805±0.136 <sup>bc</sup>	0.777±0.291 <sup>bc</sup>
F 值		43.13	21.31
P 值		<0.001	0.004

注:与正常组比较,<sup>a</sup> $P < 0.05$ ;与蓝光组比较,<sup>b</sup> $P < 0.05$ ;与溶剂组比较,<sup>c</sup> $P < 0.05$ (单因素方差分析,LSD-*t*检验) NF-κB:核因子 κB;p-NF-κB:磷酸化核因子 κB

Note: Compared with normal group,<sup>a</sup> $P < 0.05$ ; compared with blue light group,<sup>b</sup> $P < 0.05$ ; compared with solvent group,<sup>c</sup> $P < 0.05$  (One-way ANOVA, LSD-*t* test) NF-κB: nuclear factor-κB; p-NF-κB: phosphorylation of nuclear factor-κB

### 3 讨论

MGD 是眼科常见病,以老年患者居多<sup>[6]</sup>。目前 MGD 的发病机制尚不完全明确,但有不少研究证实慢性炎症在 MGD 中起着中心环节的作用<sup>[9-10]</sup>。产生慢性炎症的诱因包括眼部因素、年龄、免疫系统疾病、用药因素和环境因素等。慢性炎症导致睑板腺功能异常,睑板腺开口阻塞。睑板腺开口阻塞是 MGD 出现的标志之一,往往同时伴随睑板腺脂质堆积、睑板腺腺体萎缩、睑缘鳞状上皮化生等一系列病理改变。因此,如何有效控制慢性炎症反应是预防和治疗 MGD 的关键。

目前,MGD 的主要治疗方法包括热敷、睑缘清洁、局部用药和全身用药等。近年来,中药眼贴等中药制剂也被逐渐用于 MGD 的辅助治疗,并取得良好的疗效<sup>[7,11-12]</sup>,但其作用机制仍不明确。有研究表明,中医疗法对于慢性炎症性疾病的治疗有其独特优势,在中国古代医学著作,如《伤寒杂病论》《金匱要略》《温病条辩》《眼科龙目论》等中均有记载野菊花、丹参、黄柏等药材有降低体内燥热、虚火等功能。目前大量的研究也表明,野菊花、丹参、黄柏、黄连、薄荷的提取物具有较强的抗炎作用<sup>[13-15]</sup>;丹参酮 II A 已被证明可通过抑制 TLR4/NF-κB 信号通路和 Nrf2 信号通路,减少炎症因子的表达<sup>[13,16-17]</sup>;黄柏和薄荷是常用的祛火抗炎药物<sup>[18]</sup>;黄连可抑制环氧合酶 2 (cyclooxygenase-2, COX-2) 和 COX-5 的生成,从而起到抗炎作用<sup>[14,19]</sup>。

复方野菊花眼贴是在传统眼贴中加入野菊花、丹参、黄柏、黄连、薄荷等祛火抗炎中药,其主要成分为野

菊花和丹参,是按照现代工艺所制成的一种新型中药眼贴。复方野菊花眼贴在减轻眼部慢性炎症中已有不少应用,且疗效较好<sup>[8]</sup>。目前,野菊花类眼贴对 MGD 治疗效果尚不清楚,且既往研究多集中在当 MGD 较为严重时进行治疗。因此,本研究通过预防实验和治疗实验探讨复方野菊花眼贴对 MGD 的影响及其作用机制。

本研究中采用高能短波蓝光致小鼠睑板腺功能异常模型。已有研究表明,蓝光暴露所产生的慢性损失可导致眼部慢性炎症的产生,从而导致结膜炎、视网膜病变、白内障、神经退行性相关病变等<sup>[20-23]</sup>。雄性 12~15 周龄 C57BL/6J 小鼠在光照度为 2 000 lx、波长 460 nm 的蓝光作用下,每天暴露 6 h,15 d 后即可出现睑板腺开口阻塞,睑酯呈混浊黏稠牙膏状,下睑睑板腺发生萎缩,与临床诊断要求相符,证明睑板腺功能异常模型造模成功。在前期实验中发现,随着在蓝光中暴露时间的延长,小鼠睑板腺的萎缩基本上都是从睑板腺下睑开始,可能与小鼠睑板腺形态有关,即小鼠睑板腺下睑普遍小于上睑,其睑板腺导管及睑板腺开口普遍更小,更容易发生阻塞,进而引起小鼠睑板腺腺体萎缩。

在预防实验中,眼贴组小鼠睑板腺开口阻塞程度和睑板腺下睑剩余面积指标优于溶剂组;而在治疗实验中,眼贴组的小鼠睑板腺开口阻塞程度和睑板腺下睑剩余面积指标较溶剂组基本无变化,提示复方野菊花眼贴在睑板腺功能异常早期对小鼠睑板腺有一定的保护作用。在预防实验和治疗实验中,睑板腺油红 O 染色发现,蓝光组、溶剂组、眼贴组中均无大量脂质沉积,可能是睑板腺开口阻塞后引起睑板腺腺泡中大量脂质的堆积需要一定时间,同时也证明了本实验所用模型为轻度睑板腺功能异常模型。本研究中苏木精-伊红染色结果可见,预防实验和治疗实验的眼贴组睑板腺腺体完整,结构清晰,细胞排列紧密,腺体间无炎性细胞浸润,表明复方野菊花眼贴能够有效减轻睑板腺组织的炎症损伤。本研究中实时荧光定量 PCR 结果显示,在预防实验中眼贴组的 IL-1β、IL-6、IFN-γ 和 TNF-α mRNA 相对表达量较溶剂组均有所降低;在治疗实验中,眼贴组的 IL-1β、IL-6、IFN-γ mRNA 相对表达量较溶剂组均明显降低,TNF-α 相对表达量有降低趋势,但差异无统计学意义,可能是由于治疗时间偏短,炎症的消退还需一段时间。有研究表明,MGD 是由于 NF-κB 信号通路的激活<sup>[3]</sup>,导致慢性炎症的发生。NF-κB 信号通路的激活会引起 IL-1β、IL-6、TNF-α、IFN-γ 等炎症因子表达升高,同时也有研究表明 TNF-α 的升高会激活 NF-κB 信号通路<sup>[3,24]</sup>。本研



究结果显示,预防实验和治疗实验中眼贴组 NF- $\kappa$ B 和 p-NF- $\kappa$ B 相对表达量均低于溶剂组,说明复方野菊花眼贴能够改善睑板腺功能,可能与抑制了 NF- $\kappa$ B 信号通路有关。然而,究竟是眼贴中哪种具体有效成分发挥了作用仍需进一步研究。

综上所述,本研究通过预防实验和治疗实验观察了复方野菊花眼贴对早期 MGD 的作用效果,结果发现其可通过抑制 NF- $\kappa$ B 信号通路,降低睑板腺组织中 IL-1 $\beta$ 、IL-6、TNF- $\alpha$ 、IFN- $\gamma$  等炎症因子的表达,保护睑板腺组织,改善睑板腺功能。本研究结果为 MGD 的药物干预研究提供了新的思路。

**利益冲突** 所有作者均声明不存在利益冲突

**作者贡献声明** 李勇:参与研究设计、研究实施、收集数据、分析/解释数据、起草文章、论文修改;黄彩虹、李清坚、王玉倩:实施研究、收集数据、分析/解释数据;吕雨霏、张兆强、胡皎月:参与研究选题、分析/解释数据、起草文章;刘祖国:参与研究设计、论文内容修改和审核、最终定稿

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