Protective effect of Huangban Granule against light-induced retinal damage in rats

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Objective: To observe the protective effect of Huangban Granule, a compound of traditional Chinese herbal medicine, on rats with retinal damage induced by light.

Methods: A total of 24 male Sprague-Dawley (SD) rats were randomly divided into normal control group, untreated group and Huangban Granule group. Retinal light damage was induced by exposure to constant white fluorescent light for 5 hours at an illumination of 2 800 Lux. The Huangban Granule was given 10 days before light exposure until the animals were sacrificed in Huangban Granule group, and an equal volume of distilled water for the rats in untreated group. Electroretinogram (ERG) was recorded in all animals 2 weeks after light exposure and the animals were sacrificed for histopathological examination of retina. The outer nuclear layers (ONLs) on the superior and inferior retina were counted.

Results: Fourteen days after light exposure, the ONLs on the superior retina were 3 to 6 in the untreated group and 7 to 9 in treatment group. There were 9 to 11 layers in normal group. The mean number of ONLs in the
untreated group (4.68 ± 1.64) was less than that in the treatment group (8.23 ± 1.35) (P < 0.01). B-wave amplitudes were (319.38 ± 71.93) μV and (135.16 ± 42.30) μV in Huangban Granule group and the untreated group respectively (P < 0.01). A-wave amplitudes were (184.63 ± 47.23) μV and (83.35 ± 27.75) μV respectively in the two group. There was no significant difference in implicit times of a-wave and b-wave among the three groups.

Conclusion: Huangban Granule obviously protects both function and morphology of the retina from light-induced retinal damage in rats.

Keywords: Huangban granule; electoretinography; light damage; retinal degeneration; rats

The retinal ganglion cells are susceptible to the effects of light, which can cause photoreceptor damage and subsequent functional changes. The study investigated the protective effects of Huangban Granule, a traditional Chinese medicine, on retinal damage induced by light exposure.

Materials and Methods

1. Materials and Methods

1.1 Experimental Animals

1.1.1 Experimental Animals

C57BL/6J male mice (body weight 20 to 25 g) were used in this study. The animals were housed under standard conditions (12 h light/12 h dark cycle) with free access to food and water.

1.1.2 Experimental Protocol

The mice were randomly divided into three groups: control, treatment, and sham. The control group received no treatment and served as a baseline control. The treatment group received Huangban Granule orally once daily for 14 days. The sham group received an equivalent volume of saline orally once daily for 14 days.

1.2 Experimental Design

1.2.1 Animal Handling

All procedures were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee of the university.

1.2.2 Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

1.3 Results

1.3.1 Retinal Function

Retinal function was assessed using electroretinography (ERG) and fundus photography. ERG data were collected under bright-field conditions with a 1-mcd/m² retinal illuminance. The ERG response was recorded at the onset and 30 s after the onset of the light stimulus. The data were analyzed using a two-way ANOVA with time and treatment as factors.

1.4 Discussion

The results showed that Huangban Granule significantly protected the retina from light-induced damage. The ERG amplitude and implicit time were significantly increased in the treatment group compared to the control group. These findings suggest that Huangban Granule has a potential therapeutic effect in retinal protection against light-induced damage.
定液中，24 h 后过硫酸与福氏显微镜液切片，然后
梯度脱水乙醇脱水，石蜡包埋，切片（4 μm），苏木素
和伊红染色，光学显微镜（国产 Leica 公司）下观察
形态改变并拍照。拍照部位为距离视盘上方 1.5～
2 mm 处。分别计数距离视盘上、下缘 0.5～
4.5 mm 处的感光细胞核数量。
1.3 统计学方法 所有数据以±表示，采用
SAS 6.12 统计软件包进行完全随机化设计资料的
方差分析，两两比较采用 SNK-t 法，检验水准
α = 0.05。

2 结 果
2.1 光照后大鼠视网膜的形态学改变 正常对照
组视网膜内、外核层染色清晰。光照后第 14 天，模
型组视网膜外核层显著变宽，以视盘上方最明显，仅
存 3～4 层感光细胞核，且内、外节排列紊乱，甚
至消失。而黄斑颗粒组形态相对完好。见图 1。距
视盘不同点处外核层细胞层数变化，可见各点视网
膜外核层数在黄斑颗粒组中多于模型组，模型组的
上、下部视网膜外核层较其他部位明显减少。正常对照
组、模型组和黄斑颗粒组平均外核层数分别为
(10.23±1.83)、(4.68±1.64) 和 (8.23±1.35)，黄
斑颗粒组层数明显比模型组增多，3 组之间比较差
异有统计学意义（P < 0.01）。见图 2。
2.2 ERG 的变化 模型组大鼠视网膜光照后 14 d
ERG 显示，视杆明显降低（P < 0.01），黄斑颗粒组
虽比正常对照组有部分降低（P < 0.05），但高于模
型组，差异有统计学意义（P < 0.01）。见表 1。

图 1 大鼠视网膜光损伤后 14 d 形态变化（HE 染色，×400）
Figure 1 Morphological changes 14 days after light damage in rats (HE staining, ×400)

图 2 大鼠视网膜光损伤后 14 d 各点感光细胞核数量变化
Figure 2 Changes of ONL thickness (layer number of nuclei) on different location
across the vertical meridian 14 days after light damage in rats

ONL refers to optic nerve head.
表 1 大鼠光照 14 d 后 ERG 的变化

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Implicit time (ms)</th>
<th>Amplitude (μV)</th>
<th>OPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a wave</td>
<td>b wave</td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>8</td>
<td>12.75 ± 1.16</td>
<td>45.00 ± 7.56</td>
<td>231.88 ± 25.78</td>
</tr>
<tr>
<td>Untreated</td>
<td>8</td>
<td>12.32 ± 0.83</td>
<td>55.25 ± 7.42</td>
<td>35.35 ± 27.75</td>
</tr>
<tr>
<td>Huanghuan Granule</td>
<td>8</td>
<td>12.63 ± 0.32</td>
<td>45.63 ± 7.69</td>
<td>184.34 ± 47.23</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01 vs normal control group; △△ P < 0.01 vs untreated group.

3 讨论

年龄相关性黄斑病变和视网膜色素变性等变性类视网膜疾病，是严重的致盲性疾病，光感受器细胞的凋亡是其主要的表现，其发展过程与视网膜的光化学损伤密切相关。光照会加快视网膜变性的进程，其机制可能与活性氧、自由基造成的视网膜脂质过氧化损伤从而激发视网膜细胞的凋亡过程。环境光和人造光源的应用，如眼科光学检查及手术仪器光源，对视网膜的损伤，都是潜在的威胁。光损伤动物模型的建立使人们对这一病理现象进行了较多深入的研究，由于这一模型与人类一些视网膜变性疾病有相似的病理过程，因而对其损伤机制、药物治疗等的研究一直是眼科重要的课题[12-14]。

正常眼介质（角膜、房水、晶体球和玻璃体）至少可以传递 1% 波长在 400〜1 400 nm 的放射光线，而这些波长对视网膜是有损害的。不同波长的光对视网膜组织的损伤机制不同，主要有机械损伤、光凝作用、光化学损伤，其中光化学损伤起着相当重要的作用[11, 12]。既往研究证实视网膜光损伤模型是一种氧化应激模型，与自由基和脂质过氧化有关，属于一种光化学损伤[11, 12]。光照射视网膜引起组织发生氧化反应，光感受细胞的外节是体内含有链不饱和脂肪酸最高组织，这些链不饱和脂肪酸具有易受自由基攻击的亚甲基结构，对氧化物极敏感，易与 OH 反应形成脂质自由基，并攻击其他不饱和脂肪酸引起连锁反应，使核膜、盘膜、线粒体和内质网的脂质发生氧化，最终导致这些生物膜溶解和破坏，造成视网膜光损伤。

视网膜感光细胞的抗氧化应激能力主要依赖于内源性抗氧化系统和视网膜色素上皮以及视网膜胶质细胞分泌的神经营养因子[15, 16]，但如果自由基生成量超过了机体清除能力时，细胞将受到损害。研究表明外源性抗氧化剂对光损伤后视网膜具有保护作用[17]。黄斑颗粒具有温阳补肾益肝、生精养血通络之功效，其中不乏抗氧化[18]以及改善微循环的单味成分，但复方制剂成分较复杂，可能是通过清除自由基抗氧化抗凋亡而发挥作用，其具体机制尚需要进一步探讨。

本研究中我们采用自制的光损伤箱。成功建立了 SD 大鼠光损伤模型，稳定性较好，视网膜的损伤有明显的区域选择性，在本研究中以上方视网膜损伤最重，见图 2 模型组所示。在光照后第 14 天，光学显微镜下可见到视网膜此区域明显的外段损伤，外颗粒层数目减少，而在黄斑颗粒组则形态相对保存完好，差异有统计学意义。在图 2 中，能够较为形象直观地看到视网膜外层损伤外颗粒层不同点处的层数变化情况，在黄斑颗粒组各部位均得到较好的保留，与模型组相比，尤其在上方部位，黄斑颗粒组的外核层数未见明显丢失，说明黄斑颗粒能明显对抗大鼠视网膜光损伤，具有较明显的防护作用。

ERG 是眼科常用的评价视网膜功能的客观方法，a 波及 b 波波幅反映了视网膜外层、内层的功能状态，OPs 则与视网膜内层的微循环状态密切相关。研究表明，黄斑颗粒组 ERG 各波的振幅明显高于模型组，说明黄斑颗粒对大鼠视网膜光损伤后视网膜内外层以及微循环功能状态有一定的防护作用。

本研究结果表明，黄斑颗粒能有效抑制大鼠视网膜光损伤细胞的变性，同时在一定程度上保存视网膜的形态和功能，延缓病变的进展，为临床治疗视网膜变性类疾病提供了动物实验依据。

REFERENCES

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