EFFECT OF LIGHT STIMULATION ON THE DISTRIBUTION OF N-METHYL-N-NITROSOUREA-INDUCED RETINOPATHY

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Abstract: For determination of the effect of light stimulation on the distribution of the lesions in N-methyl-N-nitrosourea (MNU)-induced retinopathy, MNU-treated (70mg/kg) rats with sutured closed right eyelid were housed under constant light (150 lux for 24 hours). In spite of the unilateral eyelid closure, retinopathy was observed in bilateral retinas by 24 hours after MNU treatment and the retinal lesion was severe in the area centralis. In addition, the retinopathy was more pronounced in the non-closed left retina than that in the closed right retina. In the morphometrical analysis of the retina at 21 days after MNU treatment, the ratio of the outer nuclear layer thickness to the whole retinal thickness was markedly reduced in the area within 2 mm distance from the optic nerve head in both retinas. These results indicate that the light stimulation does not critically affect the distribution of the MNU-induced lesions but does enhance the severity of the lesion. (J Toxicol Pathol 9: 85~89, 1996)

Key words: Retina, Retinopathy, MNU (N-methyl-N-nitrosourea), Effect of light stimulation, Rat

Introduction

N-methyl-N-nitrosourea (MNU) is a potent alkylating agent that, when given to laboratory animals, induces retinal lesion caused by alkylating DNA damage in photoreceptor cells. Recently, we reported that the MNU-induced retinopathy in rats housed in a 12-hour light-dark cycle was extremely pronounced in the area centralis. However, the reason that the MNU-induced retinopathy is limited to the area centralis is still unknown.

Light-induced retinopathy has been reported to be more severe in the area centralis than in the periphery. It is also unknown whether the light stimulation influences the regional distribution of the MNU-induced retinopathy.

Thus, the present study was carried out to determine whether the distribution of the MNU-induced retinopathy is related to constant light stimulation. In this report, we provide histopathological data regarding the MNU-induced retinopathy in rats housed under 24-hour lighting condition after closure of the unilateral eyelid, together with the measurements of the thickness of the outer nuclear layer (ONL) and whole retina.

Materials and Methods

Twenty-four 3-week-old male Sprague-Dawley (SD) rats from SLC Japan Inc. (Shizuoka, Japan) were used in this study. The right eyes of all 24 rats were closed by lids suturing under ether anesthesia. The rats were then housed in constant light environment with the in-cage illumination level of about 150 lux. The room temperature was maintained at 23±1°C and food and water were provided ad libitum.

Seven days after lid suturing, 70 mg/kg body weight of MNU (Nacalai Tesque) was dissolved in physiological saline and given once to each of 12 rats via the intraperitoneal route. As a control group, the other 12 rats received physiological saline.

Two rats in each of the two groups were killed according to the following schedule: 1, 2, 3, 7, 14, and 21 days after MNU or physiological saline treatment. Each rat was given an excess dose of ether, and bilateral eyes were removed and pre-fixed by immersion in Davidson's solution for 30 minutes. After prefixation, the eyes were post-fixed in 10% neutral buffered formalin for 24 hours. They were...
cut on the anterior–posterior axis and sectioned from the central retina, dehydrated, and embedded in paraffin. The sections including the optic nerve head (ONH) were stained with hematoxylin and eosin.

For definition of the regional distribution of the MNU-induced retinal changes, the thickness of the retinas from the rats killed 21 days after MNU treatment was measured. The ONL thickness and whole retinal thickness were estimated for each retina with an image analyzer (LUZEX 2D, Nicon Co.) at ×50 magnification. The ratio of the ONL thickness to the whole retinal thickness \[ \text{ONL/(whole retina-ONL) \times 100} \] was calculated. The eye sections were divided into eight segments of 1 mm interval beginning at the ONH and progressing to the ora serrata (Fig. 1). The retinal and ONL thickness were measured at each of the 8 points, and the average value was calculated for each pair of values for points located equidistant from the ONH (1 and 1', 2 and 2', 3 and 3', 4 and 4').

**Results**

The microscopic results are summarized in Table 1.

*Retinal histopathology in the MNU-treated rats*

At 24 hours after MNU treatment, disarrangement of the ONL cells appeared in the non-closed left retina and the closed right retina. Sequential obser-
Table 1. Summary of Microscopic Observation

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>MNU-treated group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left retina*</td>
<td>Right retina**</td>
</tr>
<tr>
<td></td>
<td>Disarrangement of outer nuclear cells</td>
<td>Disarrangement of outer nuclear cells</td>
</tr>
<tr>
<td>2</td>
<td>Mild diminution of ONL thickness</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>Severe diminution of ONL thickness</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>Disappearance of ONL</td>
<td>Wavy arrangement of ONL</td>
</tr>
<tr>
<td>21</td>
<td>Disappearance of ONL and vacuole formation in the inner nuclear and inner reticular layer</td>
<td>Severe diminution of ONL thickness</td>
</tr>
</tbody>
</table>

*: Left retina removed from the eye with non-closed lid.
**: Right retina removed from the eye with closed lid.
—: No changes
Fig. 4. Retinal degeneration is seen in the area centralis (C) of a non-closed eye, 21 days after MNU treatment. The boundary between the area with and without degeneration is distinguished. HE, ×100.

viation of the left retina revealed the following changes: The ONL thickness was decreased at 3 days after MNU treatment, and at 14 days, the ONL had disappeared. At 21 days, some vacuoles had formed in the inner nuclear and inner reticular layer (Fig. 2).

In the right retina, the ONL cells showed disarrangement, and the ONL showed wavy arrangement at 14 days after MNU treatment. At 21 days, the thickness of the ONL was decreased (Fig. 3).

In spite of unilateral eye closure, bilateral retinas showed degeneration in the area centralis. The boundary between the areas with and without degeneration could be distinguished in both retinas (Fig. 4).

Retinal histopathology in control rats

At 3 to 21 days after physiological saline treatment, the thickness of the ONL was slightly decreased in the left eye, whereas the right retina showed no significant changes.

Retinal morphometry in the MNU-treated and control rats

The ratio of the ONL thickness to the whole retinal thickness is shown in Fig. 5.

In the retina of the non-closed eye in the MNU-treated rats, the ratio is estimated as 0 in the area within 2 mm distance from ONH, because the ONL has absolutely disappeared in that area. However, in the periphery (near the ora serrata), the ratio shows almost a normal value. The ratio markedly changes at the region which is from 2 to 3 mm distance from ONH.

In the retina of the closed eye in the MNU-treated rats, the ONL thickness was reduced in the area centralis as compared with the periphery. The ratio in the periphery remained almost normal.

In the non-closed retina in the control rats, there was mild decrease of the ONL thickness throughout the retina.

Discussion

Previously we reported that the MNU-induced retinopathy in rats housed in cyclic light environment was extremely pronounced around the ONH. This observation indicates that the characteristic distribution of retinal change might be attributable to the regional vulnerability to light. Thus, in this study, the effect of constant light stimulation on the MNU-induced retinopathy was examined.

Even in the control rats, a reduction of the ONL thickness was observed in the retina of the non-closed
eye. This result indicates that the retinal changes occur at the present illumination level (150 lux, 24 hrs). To date, light-induced retinopathy has been considered to be related to photochemical reaction or increase in retinal temperature. The rats used in this study may be susceptible to the light-induced retinopathy because albino rats have no iris pigmentation which lowers the light intensity. The observation in the control rats indicates that constant light stimulation affects the histological features of the non-pigmented retina.

Twenty-one days after the MNU treatment, the ONL thickness in the retina in the closed eye showed reduction whereas that in the non-closed eye the ONL had disappeared. This observation indicates that the MNU-induced retinopathy is augmented by light stimulation.

The present experiment was carried out to examined the hypothesis that the MNU-induced retinal lesion around the ONH might be reduced by light interception. The area centralis, however, was degenerated regardless of whether the eye was closed or not. Since the photoreceptor cells are distributed widely throughout the retina, if only alkylation of the photoreceptor cells were responsible for the MNU-induced retinopathy, the lesion would extend throughout the retina. However, in the present study the area centralis of the retina was found to show selective injury, suggesting that some other mechanism may be related to MNU-induced retinopathy.

In summary, MNU-induced retinopathy was observed in the area centralis whether or not the eye was sutured closed. The area within 2 mm distance from the ONH showed particularly injury. The comparison between non-closed and closed retinas revealed that the light stimulation is related to the MNU-induced retinopathy, although the light stimulation does not critically affect the regional distribution of the lesion.

References