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13. ABSTRACT *(Maximum 200 Words)*

Purpose: Laser induced retinal injuries are operational and medical problem in the battlefield as a result of lasers used in military equipment and the introduction of visually incapacitating laser weapons. It is not possible to prevent all these injuries and there is no treatment. This study was designed to evaluate the neuroprotective effect of dextromethorphan, which is FDA approved and clinically used drug, in our rat model of laser-induced retinal-lesions.

Methods: Argon laser retinal lesions were inflicted in the eyes of 36 pigmented rats. The treated group received dextromethorphan 50 mg/kg dissolved in saline, immediately after exposure to laser and then 20 mg/kg every 8 hours for 3 days. The control group received the solvent at the same volume and schedule. The rats were sacrificed 3, 20 or 60 days later and the retinal lesions were evaluated by light microscopy and morphometric measurements.

Results: No difference was demonstrated between the treated and the control groups, in the histopathologic appearance of the lesions, their size and the magnitude of photoreceptor nuclei loss within the lesions.

Conclusions: Dextromethorphan treatment is not effective in ameliorating the retinal injury induced by argon laser in rats, when given systemically at the dose and schedule evaluated.

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Neuroprotection, laser, retina, dextromethorphan, rat

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FOREWORD

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M. Roane 31.10.1999

PI - Signature Date

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INTRODUCTION:

Extensive research has focused on protective devices and safety measures to prevent laser-induced retinal injuries, as well as on their treatment. Unfortunately, there is yet no accepted therapy for reducing retinal neuronal death and the associated scarring and disruption of the retinal architecture resulting from exposure to laser radiation. Neuronal lesions tend to spread after an injurious event such as trauma or ischemia, and neuroprotective compounds can minimize this spread and thus limit further damage [1,2]. We have shown that this is the case in laser-induced retinal lesions as well. Consistent experimental evidence points to the efficacy of non-competitive NMDA antagonists in animal models of CNS global or focal ischemia [4-7]. Many of these pharmacological compounds are currently undergoing clinical trials for treatment of stroke and traumatic brain injury. One of the most promising is dextromethorphan [8,9], a dextrorotatory opioid derivative with both calcium channel and NMDA ion-channel blocking properties [10-12]. It was approved for use as an antitussive for many years before its recognition as an NMDA blocker, and it is now in the second phase of clinical trial for traumatic brain injury and neurosurgical prophylaxis. In the current research we evaluated the neuroprotective effectiveness of dextromethorphan, known for its safe usage in healthy human subjects and patients, in our well-established rat model for retinal lesion following argon laser lesions [3,13-16]. Retinal lesions were induced by argon laser irradiation in retinas of pigmented rats. Thirty-six rats were used for the evaluation of the drug. Eighteen were treated by dextromethorphan and eighteen (the control group) receives the vehicle-saline. The rats were sacrificed at 3, 20 and 60 days after exposure to laser. The efficacy of dextromethorphan treatment in limiting the extent of the injury was evaluated histologically at the acute, intermediate and late phases after injury. The evaluation included light microscopic examination of serial sections, morphometric measurements of the lesion diameter and assessment of the extent of photoreceptor cell loss in the retinal outer nuclear layer.

BODY:

Methods

Animals

Pigmented DA rats (Strain DA/OLa/Hsd, Harlan OLAC Ltd., Blackthorn Bicester Oxon., England; raised in Tel-Aviv University animal house), 90 days old, were used for the experiments. The posterior segment of the eye of this strain has a uniform pigmentation, making it particularly useful for retinal laser injury production. The animals were fed ad libidum with a normal diet and maintained on a 12-h light/dark cycle. They were anesthetized by intraperitoneal injections of ketamine (40 mg/kg) and xylasine (8 mg/kg). Laser retinal lesions were produced in each eye, and the rats were sacrificed after 3, 20 or 60 days by lethal doses of pentobarbital sodium injected intraperitoneally. The eyes were enucleated for histopathologic and morphometric evaluations.

All procedures involving animals were performed according to the guidelines of the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

Laser injury

Following dilatation of the pupil with topical topicamide 0.5% sterile drops (Mydramid, Fischer), a contact lens, specially constructed in our laboratory to fit a rat eye, were coupled to the cornea with 2.5% hydroxypropyl methylcellulose. Six argon laser (Novus 2000, Coherent, Palo Alto Ca) lesions (514 nm, 200 μm, 0.1 W, 0.05 sec) were produced in each eye, one to three disc diameters from the optic disc. These laser settings were found in our previous studies to result in lesions of uniform size and configuration, involving mainly the outer retinal layers.

Administration of drugs

The treated group received dextromethorphan (d-3-Methoxy-N-methylmorphinon, Sigma Chemical Co., St Louis Missouri) 50 mg/kg dissolved in saline, immediately after exposure to laser and then 20 mg/kg every 8 hours for 3 days. The control group received the solvent at the same volume and schedule.

Experimental design

A total of 36 rats were used in this study. Six laser lesions were produced in each eye of each animal. Half of them (18 rats) served as the test (treated) group, which received intraperitoneal injections of dextromethorphan dissolved in saline and the other half served as the control group and received intraperitoneal injections of the vehicle-saline at the same regimen. The effect of the treatment was evaluated at three time points: 3, 20 and 60 days after the injury was inflicted. The retinal lesions were evaluated for histopathologic and morphometric differences in a masked fashion.

Histopathologic and morphometric studies

The rats were sacrificed 3, 20 or 60 days after irradiation. The eyes were enucleated and fixed in 2% glutaraldehyde. Using a surgical microscope, the posterior segment of the fixed eyes was dissected into tissue samples, each incorporating one retinal laser lesion.

A total of 48 laser lesions from the treated group and 48 laser lesions from the control group (4 laser lesions from each animal) were subjected to both histological and morphometric examination. The tissue samples were embedded in plastic (epon) blocks, sectioned serially (2μm) with an ultramicrotome and stained with toluidine-blue.

Stained sections from the central part of the lesion, exhibiting the greatest amount of laser-induced retinal destruction, were examined by light microscopy for histopathological changes of the retinal lesions.

To further evaluate the neuroprotective effects of dextromethorphan, a quantitative morphometric assessment of the retinal lesions were carried out using a computer-assisted image analysis system (Scan Array 2, Galai, Migdal Haemek). Two morphometric measurements were performed on each lesion in order to evaluate the severity of the argon laser injury.

The first measured the largest diameter of the lesion. This was done by determining the edges of the lesion area, according to the changes in the retinal pigmented epithelium and in the cytoarchitecture of the outer retinal layers (Figure 1). These changes included loss of pigment granules in the retinal pigment epithelium, loss of its monolayer structure and loss of retinal pigment epithelial cells, disruption of the outer and inner segments, changes in the outer nuclear layer, and thickness and infolding of the inner retinal layers (the inner nuclear layer, inner plexiform layer, ganglion cell layer and nerve fiber layer). The transition from normal retina to the lesion area is well defined, making the criteria for morphometric measurements clear and reproducible.

The second evaluated the extent (percentage) of photoreceptor cell loss. This was done by calculating the differences between the numbers of ONL nuclei in the outer nuclear layer at the area of the lesion, with that of the normal outer nuclear layer along 100 μm of the retina on both sides of the lesion.

All histopathologic and morphometric evaluations were performed in a masked fashion.

Statistical analysis

A sample size of twelve animals enables to detect any differences between the treated test groups and the vehicle-injected control groups, that is twice the standard deviation within the groups.

All morphometric measurements were performed in two sections at the center of each retinal lesion and averaged. A two-way analysis of variance was used to calculate the significance of the treatment and time effects.

Results

In both treated and control eyes, histopathological examination of the retinal lesions 3 days after laser irradiation revealed damage to the retinal pigment epithelium, the inner and outer segments of the photoreceptors, the outer nuclear layer, the outer plexiform layer and the inner nuclear layer (Figure 1, upper photographs). The retinal pigment epithelium showed local proliferation with formation of a multilayered membrane containing phagocytic cells. The outer and inner segments of the photoreceptors were disrupted and deformed. The outer nuclear layer showed loss of nuclei, as well as the presence of pyknotic nuclei at the periphery, tapering off towards the center of the lesion, where they were completely absent. The center of the lesion was filled with cellular debris, dispersed pigment granules and pigment-laden macrophages. The outer plexiform layer was disrupted and the inner nuclear layer was mildly edematous. The inner plexiform layer, the ganglion cell layer and the nerve fiber layer were folded internally, creating internal bulging at the inner retinal surface over the area of the lesion.

At 20 and 60 days after laser irradiation (Figure 1, middle and lower photographs), the multilayered proliferative membranes in the lesion became more thinned and showed occasional neovascularization. The retinal pigment epithelium layer had reformed. The outer and inner segments of the photoreceptors had reformed at the periphery of the lesion, showing disruption only in the central area. The outer nuclear layer showed fewer pyknotic nuclei, and the central area, in which there was total loss of the outer nuclear layer, had decreased in size. The inner plexiform layer was less edematous. The rest of the histopathological findings did not differ significantly from the findings at 3 days. Bulging of the inner retinal surface was still evident. This bulging was assumed to be a result of the edema of the inner retinal layers at the earlier period, with subsequent traction of the normal retinal layers at the edge of the lesion towards its center once the edema had resolved.

The histological findings of the laser-induced retinal lesions were similar to those described in the literature [17]. The histopathological findings in the lesions of the treated group did not differ from those of the control group (Figure 1). Furthermore, there were no significant morphometric differences between the two groups with respect to the diameter of the lesions (Fig. 2) or the loss of photoreceptor nuclei at the injury site (Fig. 3) when examined at 3 days, 20 days or 60 days after laser irradiation.

Discussion

Lasers assume increasing use in communication, industry and the military fields, accounting for increasing numbers of accidental eye injuries [18-25]. Concern has been growing that lasers might be used as a weapon in the future battlefield, making the eyes a major target [26-27]. Injury to retinal neurons, whether by traumatic, ischemic or other mechanisms, has generally been considered an irreversible phenomenon that cannot be halted or slowed down. There is no accepted therapy available for these devastating injuries. The retina is part of the central nervous system (CNS), and when injured might exhibit similar pathogenic mechanism to those involved in neuronal death following CNS injury [28-31]. New insights into these mechanisms have provided a theoretical basis for evaluating various pharmacological strategies to induce neuroprotection. Most of the available information on neuroprotection comes from studies of the CNS following traumatic or ischemic injury. It is now well documented that much of the post-injury tissue damage results from delayed inflammation and an autodestructive cascade of events [31-32]. Glutamate receptor antagonists are among the most intensively studied pharmacological agents for reducing neurotoxicity arising out of CNS damage [33-36]. Glutamate plays a dominant role in CNS [1] as well as retinal neurotransmission [37,38]. However, exposure of neurons to high concentrations of extracellular glutamate can lead to their death[1,30]. It is now well established that after CNS injury the damaged neurons release massive amounts of glutamate, which interacts with adjacent cells and eventually destroys them. Thus a biochemical cascade develops, in which injured neurons amplify the initial traumatic effect and cause the damage to spread to neighboring tissue, causing exacerbation of the original insult [1,30]. The neurocytotoxic action of glutamate appears to be modulated mostly by its N-methyl-D-Aspartate (NMDA) receptor [31-39]. Similar neurocytotoxic effects of glutamate, mediated through the activation of NMDA receptors, have been demonstrated in retinal neurons both in vitro and in vivo [40-42], and administration of NMDA-receptor blocker to retinal neurons in culture improved their survival following their exposure to glutamate [41,43]. Glutamate-receptor blockers also protect retinal neurons from hypoxic damage [40-42]. It therefore seems likely that the retina might respond to agents shown to have neuroprotective properties in the CNS.

We demonstrated[67] the neuroprotective effect of MK-801, the "gold-standard" and the most potent known NMDA-receptor antagonist, in argon laser-induced retinal injury. The lesions, which are located in the external retinal layers and the choroid, are reproducible and can be accurately quantified, making it possible to evaluate the potential damage-spread limiting effects of neuroprotective drugs. We followed the histopathological changes in the argon laser-induced retinal lesions for 60 days in MK-801 treated animals (3mg/kg, intraperitoneally) and compared them to those observed in laser lesions inflicted in retinas of control rats injected with saline. We also evaluated the severity of the argon laser injuries in treated and control lesions by morphometric measurement of the diameters of the lesions and the extent of photoreceptor cell losses in the retinal outer nuclear layer. At 20 days and at 60 days, the MK-801-treated lesions were found to be significantly smaller than their control counterparts. Differences in ONL thickness loss between MK-801-treated and control lesions were also highly significant and indicated that significant numbers of ONL neurons were rescued by MK-801 from the spread of the damage. On the basis of these results, we suggest that glutamate plays a key role in the spread of laser-induced retinal injury, by mediating the continuous destruction of the photoreceptors. Antagonism by MK-801 of these glutamate-induced effects significantly improves the outcome. However, the known toxicity of MK-801 precludes its further experimental use in human laser injuries involving the neural retinal elements.

In this study, we examined the effect of dextromethorphan on retinal injury induced by argon laser irradiation in pigmented rats. Dextromethorphan which is a dextrorotatory opioid derivative with both calcium channel and NMDA ion-channel blocking properties [10-12], is now in the second phase of a clinical trial for traumatic brain injury and neurosurgical prophylaxis. The regime used for rats was intraperitoneal injections of dextromethorphan 50 mg/kg dissolved in saline, immediately after exposure to laser and then 20 mg/kg every 8 hours for 3 days. However, the results of the current study show that dextromethorphan treatment, given systemically, at this dose and regimen, was not effective in ameliorating the retinal injury induced by argon laser in rats.

We recommend to continue with the original plan.

KEY RESEARCH ACCOMPLISHMENTS:

- Dextromethorphan treatment, given systemically, at this dose and regimen, was not effective in ameliorating the retinal injury induced by argon laser in rats.

REPORTABLE OUTCOMES:

– manuscripts, abstracts, presentations;	none
– patents and licenses applied for and/or issued;	none
– degrees obtained that are supported by this award;	Basic research by ophthalmologic resident
– development of cell lines, tissue or serum repositories;	none
– informatics such as databases and animal models, etc;	none
– funding applied for based on work supported by this award;	none
– employment or research opportunities applied for and/or received on experiences/training supported by this award.	none

CONCLUSIONS:

Dextromethorphan treatment is not effective in ameliorating the retinal injury induced by argon laser in rats, when given systemically at the dose and schedule evaluated.

We recommend to continue with the original plan to evaluate the effect of the approved drugs mementine and brimonidine.

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FIGURES

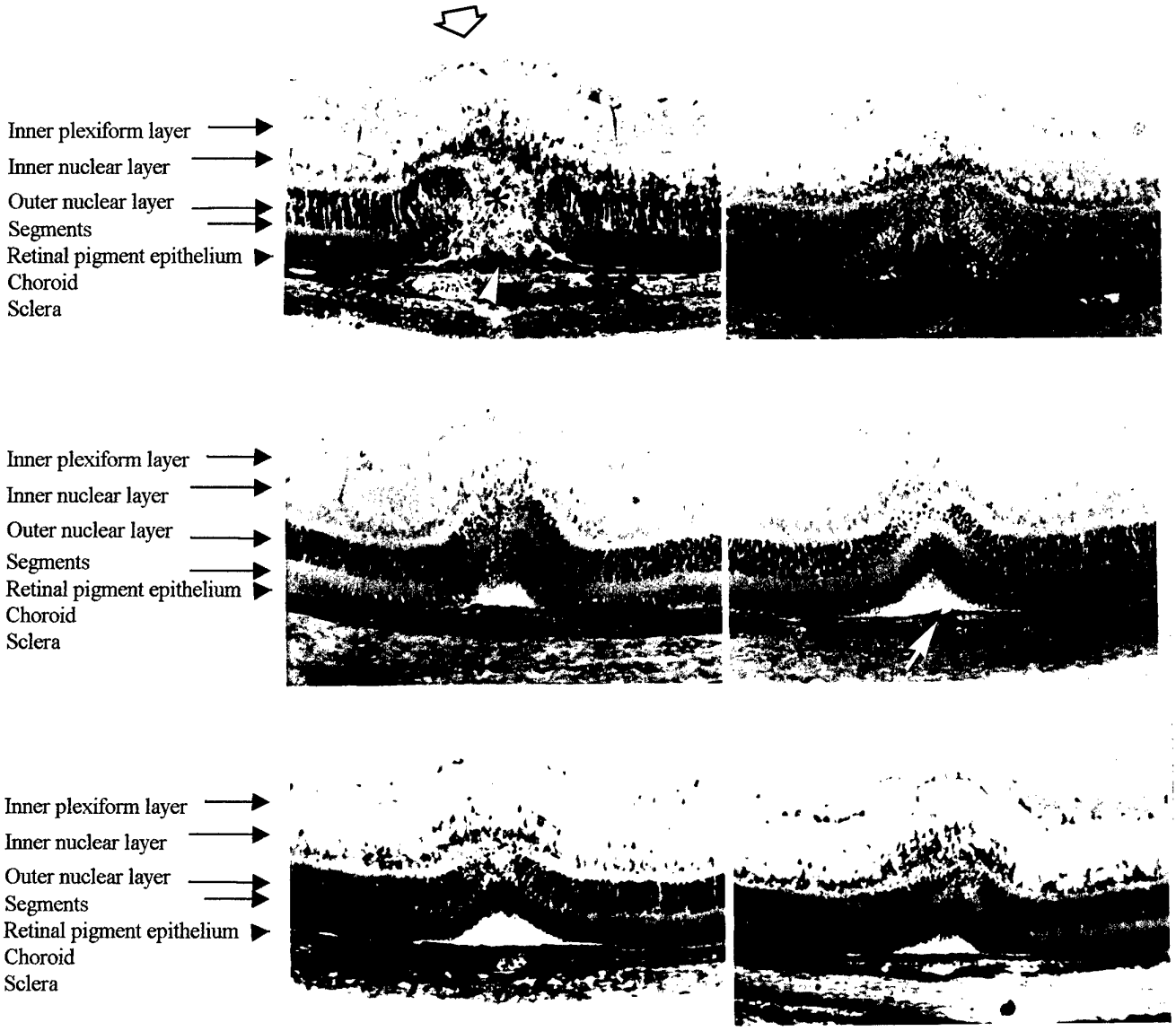

Fig. 1. Retinal lesions of control (left) and dextromethorphan treated (right) at 3 days (upper), 20 days (middle) and 60 days (lower) after laser exposure. (Toluidine blue, Original magnification x100)

Three days after exposure, the control lesion (upper left) showed disruption of the outer nuclear layer with extensive loss of nuclei at the central area of the lesion (asterisk). There was loss of inner and outer segments and development of fusiform proliferative plaque at the level of the retinal pigment epithelium (white arrowhead). An internal retinal bulging was formed at the area of the lesion (whit arrowhead). In the dextromethorphan treated rats, 3 days after exposure (upper right) similar findings are seen. Twenty and 60 days after exposure (middle and lower), thinner plaques with occasional neovascularization (white arrow) were seen. The inner and outer segments were reformed. The findings were similar in the dextromethorphan treated and the saline treated control rats.

Fig. 2. Effect of dextromethorphan on the mean retinal lesion diameter.

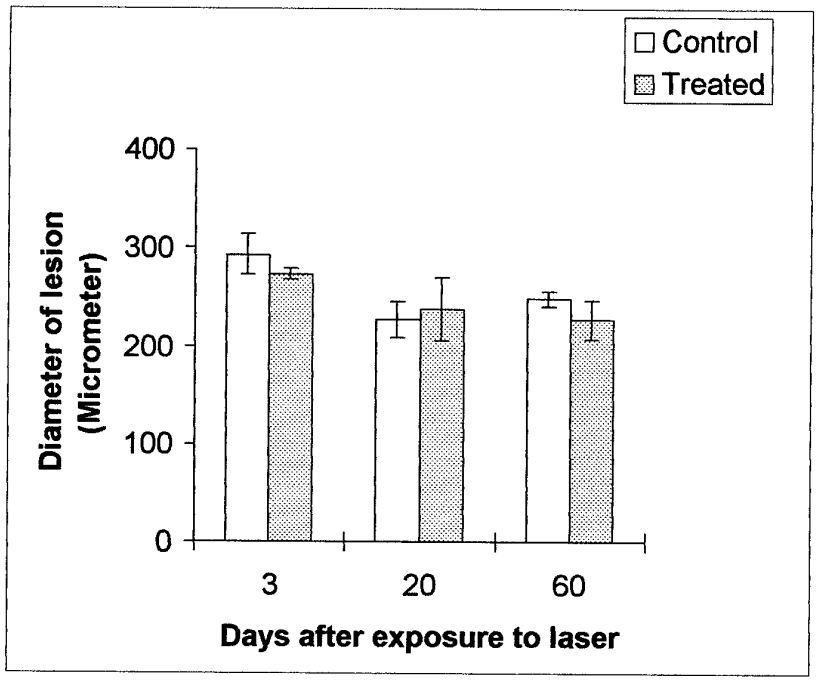

Fig. 3. Effect of dextromethorphan on the mean percentage of photoreceptor cell loss.