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The Role of miR-330-3p in UV-induced Photokeratitis: A Pilot Experimental Study

UV ile Indüklenen Fotokeratitis Üzerine miR-330-3p Uygulamasının Rolü: Pilot Çalışma

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ABSTRACT

Aim: Ultraviolet (UV) radiation-induced ocular diseases pose a growing challenge to public health. In recent years, miRNA-based therapeutics have gained attention in the treatment of ocular diseases. miR-330-3p has anticarcinogenic, anti-inflammatory, and anti-apoptotic effects. This study aimed to investigate the effects of miR-330-3p against photokeratitis following UV radiation.

Materials and Methods: Eighteen Wistar albino male rats were randomly divided into three groups (n=6): control, UV, and UV+eye drop. Chronic exposure to UV radiation was conducted for 30 days, 2 hours a day. One µL of miR-330-3p-based eye drops was applied to the UV+eye drop group once daily for 7 days. Following the treatments, eye tissues were harvested and evaluated microscopically.

Results: There was no statistically significant difference between groups in inflammation, neovascularization, epithelial proliferation, and collagen density parameters. However, the edema levels in the UV group increased compared to the control and UV+eye drop groups (all $p<0.001$). The collagen density, however, increased in the UV group and decreased in the UV+eye drop group, but the results did not indicate a significant difference ($p>0.05$).

Conclusion: miR-330-3p presents a promising treatment option for corneal damage arising from photokeratitis. Our study is the first to explore the alleviating effects of miR-330-3p in photokeratitis, yielding encouraging results.

Keywords: Ultraviolet rays, eye diseases, miRNA, ophthalmic solutions, eye drops

ÖZ

Amaç: Ultraviyole (UV) radyasyon ile indüklenen oküler hastalıklar büyüyen bir halk sağlığı sorunudur. Son yıllarda yapılan çalışmalar miRNA-tabanlı tedavilerin önemini artırmaktadır. miRNA'lardan biri olan miR-330-3p anti-karsinojenik, anti-enflamatuvar ve anti-apoptotik etkileri mevcuttur. Bu bilgiler ışığında çalışmamızın amacı UV radyasyonu sonucundaki fotokeratitise karşı miR-330-3p'nin etkilerini incelemektir.

Gereç ve Yöntem: Çalışmada 18 adet erkek Wistar Albino rat kontrol, UV ve UV+göz damlası olmak üzere üç farklı gruba ayrılmıştır. Otuz gün süresince günde 2 saat kronik UV radyasyonuna maruz bırakılmıştır. Bunu takiben 7 gün süresince UV+göz damlası grubuna 1 µL miR-330-3p içeren göz damlası uygulaması yapılmıştır. Son uygulamadan 24 saat sonra hayvanlar sakrifiye edilerek histopatolojik incelemeler için uygun koşullarda saklanmıştır.

Bulgular: Yapılan mikroskobik incelemeler sonucunda enflamasyon, neovaskülarizasyon, epitel proliferasyonu ve kolajen densitesi parametrelerinde gruplar arasında istatistiksel olarak bir farklılık belirlenmedi. Bununla birlikte istatistiksel olarak farklılık olmamasında rağmen kolajen densitesinin UV grubunda Kontrol grubuna göre yükseldiği ve göz damlası uygulamasının bunu düzenlediği görüldü. Ödem parametresi UV grubunda hem kontrol hem de UV+göz damlası grubuna göre istatistiksel olarak anlamlı şekilde yüksek olduğu ortaya koyuldu (her iki p-değeri $p<0,001$).

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Sonuç: Çalışmamızda miR-330-3p'nin fotokeratit üzerine etkileri ortaya koyulmuştur. miR-330-3p, fotokeratitten kaynaklanan umut verici bir tedavi seçeneği olarak görülmektedir.

Anahtar Kelimeler: Ultraviyole ışınlar, göz hastalıkları, miRNA, oftalmik solüsyon, göz damlası

INTRODUCTION

Ultraviolet (UV) radiation has many effects on the human body, including both beneficial and hazardous impacts. Eyes, especially the anterior sites, are the most affected by UV radiation and the most UV-sensitive part of the body[1]. Due to multiple protection methods, the cornea, retina, and epithelial compartments are exposed to macro and micro damage. There are various pharmacological aspects for alleviating corneal or retinal damage that are lacking, and further studies are still essential[2]. Growing evidence demonstrates the risk of UV exposure to corneal damage and visual impairment, which has been an emerging health care problem. Ocular exposure to UV may cause several detrimental effects, as well as loss of vision. It has been reported that corneal tissues absorb almost 80% of the UV light directly. Depending on age-related differences, during late phases of life, the absorption levels increase and have detrimental effects on the cornea and ocular layers. People should be aware of the long-term UV exposure and use protective tools. The UV radiation-induced pathophysiological cascade includes photokeratitis, oxidative stress, edema, and apoptosis of epithelial cells[3]. The ocular fluid and layers include multiple antioxidant substances that may protect the cornea and surrounding ocular tissues. High exposure to UV radiation has negative impacts on ocular tissues and fluid through the increase of corneal fluid level and photo absorption. Photokeratitis is a painful condition and is mostly attributed to corneal damage following long-term exposure to UV. The loss of epithelial cells in the superficial layers of the cornea triggers edema and vision impairment[4]. Edema is initially identified as an adaptation and prevention from UV exposure; however, the exposure level is the main determinant of the corneal damage[5]. Non-pharmacological treatment options mostly include ice application or resting in a dark place. However, in serious cases, pharmacological therapies might be essential to reduce edema and inflammatory response to the UV exposure-induced corneal damage. Over the past three decades, gene-based therapies have gained attention in most pathological conditions. MicroRNAs are small non-coding RNAs and regulate tissue-specific RNA transcription, which mostly depends on the degradation of expressed proteins[6,7]. miRNAs were involved in several cellular responses, like the cellular cycle, inflammation, and apoptosis. It was reported that over two thousand miRNAs have been expressed in the human body, and yet most of them have not been identified about their functionality. The clarification of miRNA functions has the potential to provide promising adjunctive therapies for multiple disorders. Recent studies declared that there are crucially important miRNAs for the prevention of corneal damage[8-10]. These suggestions mostly originated from experimental diabetes studies and have become apparent. miR-330-3p is a miRNA cluster that is mostly associated with anti-aging, anti-apoptotic, and anticarcinogenic effects. miR-330-3p was used in several types in a variety of studies, including rheumatoid arthritis, osteosarcoma, and melanoma, with promising alleviating effects[11-13]. Therefore, in this study, we aimed to investigate the effects of miRNA-based eye drops (miR-330-3p) on UV exposure-induced corneal damage.

MATERIALS AND METHODS

Preparation of miR-330-3p-based Eye Drop

For the preparation of the eye drop, miR-330-3p mimic was purchased from (Med Chem Express Cat No: HY-R03031, Lot no: 326920) and encapsulated with lipofectamine 2000TM. For the encapsulation, lipofectamine and miR-330-3p were incubated for fifteen minutes according to the manufacturer's protocol. The eye drops were applied 1 µL to the UV+miR-330-3p group bilaterally after 30 days of UV radiation for 7 days, once a day[14,15].

Animals

The present study included eighteen Wistar albino male rats (7-8 months; G*Power: n ≥18) purchased from Çanakkale University Experimental Research Application and Research Center, with ethical approval from Çanakkale University Animal Experiments Local Ethics Committee (decision no: 2024/04-02, date: 18.04.2024). Rats were housed individually at standard humidity and temperature (45%-50% humidity and 22±2 °C), with a 12-h dark/light cycle and fed standard pellets and water ad libitum.

Experimental Groups and Procedure

The purchased rats were randomly divided into three groups (n=6) as control, UV, and UV+eye drop. The Control group was not exposed to UV radiation and did not receive any treatment. UV and UV+eye drop groups were exposed to 2 hours of daily UV radiation for 30 days at a dose of UV-A 12.5 J/cm^2 and UV-B 0.22 J/cm^2 [16,17]. At the end of the 30 days, the UV+eye drop group was administered with the miR-330-3p-based eye drop. The UV group did not receive treatment.

Tissue Harvesting

At the end of 7 days of treatment, all rats underwent general anesthesia (ketamine-xylazine) for sacrifice. Following euthanasia with cervical dislocation, the whole eye tissue and optic nerves were harvested immediately and stored in 10% formaldehyde until histopathological analysis. Histopathological analysis the eye specimens were used for hematoxylin-eosin (H-E) and masson's trichrome (M-T) staining analysis for histopathological assessment. First, all specimens were fixed and processed for staining assessments. After all tissues were embedded in paraffin, 4-µm sections were then cut and stained with H-E and M-T[18-20]. H-E-stained sections were used to evaluate the edema, inflammation, neovascularization, and epithelial proliferation; and M-T-stained sections were used to evaluate the collagen density and structure.

Statistical Analysis

The data were analyzed by the SPSS program version 27.0 (SPSS, version 27, IBM of Armonk, New York, U.S.). One-way ANOVA test was used for determining differences. Post-hoc determinations were performed by Tukey HSD test for comparing the groups. Data were presented as means and standard errors. P<0.05 was considered statistically significant.

RESULTS

The histopathological analysis indicated that there were no significant changes in inflammation, neovascularization, and epithelial proliferation according to the H-E results, and in collagen density according to the M-T results. However, the edema levels in the UV group (3.00±0.00) were significantly increased compared to the Control group as shown in Figure 1 (0.00±0.00; p<0.001). Additionally, there was no statistically significant difference between the Control group and UV+eye drop group as shown in Figure 2 (0.83±0.54; p=0.178). Moreover, in the UV+eye drop group, edema levels were significantly decreased compared to the UV group (p<0.001). The collagen density, however, increased in the UV group (0.50±0.22) and decreased in the UV+eye drop group (0.16±0.16), but the results did not indicate a significant difference (p>0.05; Figure 2).

DISCUSSION

The present study mainly aimed to evaluate the effects of a miRNA-based eye drop in chronic exposure to UV radiation. Our results indicated that, in accordance with the literature, the UV exposure increased the edema levels in the UV exposure group significantly compared to the Control group. miR-330-3p-based eye drop significantly decreased the edema levels compared to the UV group. However, inflammation, neovascularization, and epithelial proliferation did not indicate any significant change. Additionally, collagen density decreased in the UV+eye drop group, but the results were not significant. The UV light penetrates the eye tissue layers, increasing the risk of eye cancer, inflammation, and visual disorders[21]. The overall eye tissues represent an adaptation to low exposure to UV radiation in daily life. However, prolonged

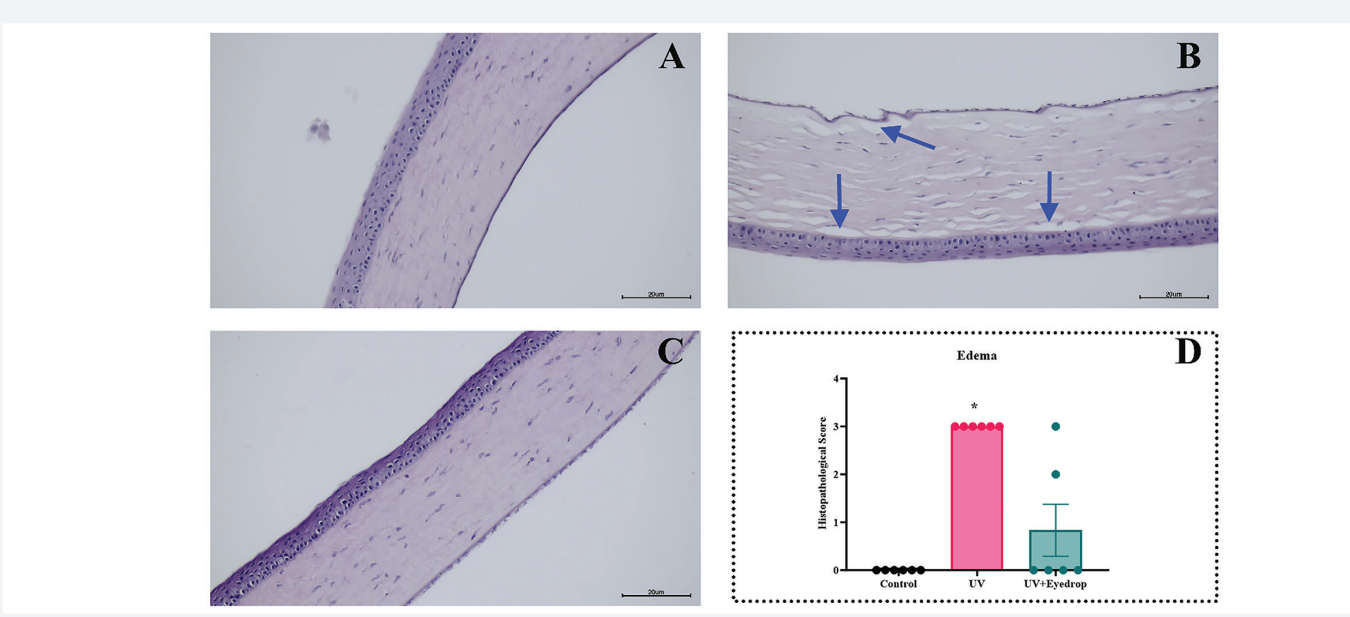

Figure 1. (A) Control, (B) UV, (C) UV+eye drop group of H-E-stained sections and (D) Histopathological scores for edema in all groups (*p<0.05 compared to the control and UV+eye drop groups)

H-E: Hematoxylin-eosin, UV: Ultraviolet

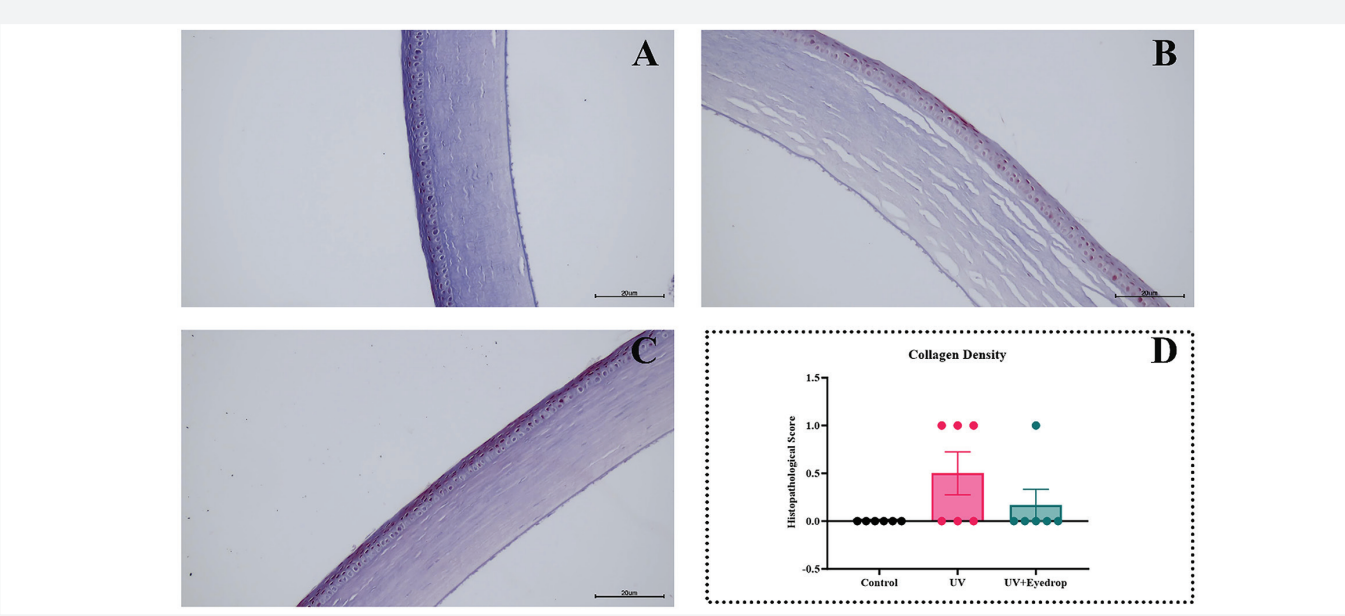

Figure 2. (A) Control, (B) UV, (C) UV+eye drop group of M-T stained sections and (D) Histopathological scores for collagen density in all groups

UV: Ultraviolet, M-T: Masson's trichrome

exposure without protection may provoke visual disorders, pain, or itchy eyes[22]. Photokeratitis is one of the major detrimental outcomes of prolonged UV exposure to the eyes. Corneal epithelial damage implicates an edema, which may also alter vision and result in clouds and haze[23]. In a study, the rats' eyes were locally exposed to the UV dose of 6.5 J/cm² daily for 5 consecutive days. Histopathological results indicated that there was a significant increase in interstitial edema and disruption of collagen structure[24]. A comparative study was established to evaluate the acute effects of 0.08 and 0.225 J/cm² UV radiation on eye tissue. It was claimed that 0.08 J/cm² UV exposure exhibited a mild edema and nerve injury, whereas 0.225 J/cm² destroyed epithelial nerves[25]. In our study, 30 days of daily 2-h exposure to UV-A 12.5 J/cm² and UV-B 0.22 J/cm² significantly increased epithelial edema levels. Considering local or acute exposure, it is suggested that daily exposure to UV radiation may also trigger epithelial disorders. Nucleic acid-based therapeutics provide promising outcomes when used for a variety of disorders, including cancer, neurodegenerative disorders, inflammatory conditions, and apoptosis-related diseases[19]. Currently, there is a wide range of disorders for which nucleic acid-based therapeutics have completed phase II studies[20]. Since the discovery of the first miRNA in 1993, miRNA-based applications have gained attention over the past decade due to their regulatory effects on transcription factors[20]. In addition to most ocular diseases, photokeratitis has become a target for miRNA-based therapeutics. In a previous study, the effects and tolerability of anti-miR-328 were assessed, and the outcomes were promising, indicating its appropriateness for phase II and phase III trials[21]. The effects of miR-127-5p in UV-exposed photokeratitis demonstrated anti-apoptotic and antioxidant effects in an *in vitro* study[22]. In an animal study on acute UV exposure eye damage, miR-129-5p eye drops reduced corneal epithelial damage and photokeratitis-induced visual loss[23]. miR-330-3p has primarily been defined as an anti-carcinogenic miRNA, exhibiting anti-inflammatory, anti-apoptotic, and antioxidant effects[24]. To date, no study exists that has investigated the effects of miR-330-3p on ocular diseases. In the present study, following chronic exposure to UV radiation, a miR-330-3p-based eye drop was applied to the rats' eyes for 7 days. Depending on chronic application, there were no statistically significant changes in inflammation, neovascularization, and epithelial proliferation. However, edema, which is the most important signal for the development of photokeratitis, was increased significantly in the non-treated UV exposure group. miR-330-3p application significantly decreased edematous levels and alleviated photokeratitis-based vision loss and haze. It is suggested that miR-330-3p might be a good candidate to reduce corneal damage based on photokeratitis following chronic UV exposure. However, further studies with acute exposure to UV light have to be established to elucidate the main molecular and effect mechanisms of miR-330-3p eye drops.

Study Limitations

There are some limitations of the study given below:

• Our study is a pilot study so lower numbers of animals were used. Further studies have already been established to elucidate the effects of chronic and acute exposure to UV radiation on photokeratitis.

• Additionally, our model was different from previous studies as the exposure of UV light has been established to the whole body representing the real exposure in daily life, not locally to the eyes.

CONCLUSION

Future Directions

Ocular diseases present challenges due to limited treatment options and the sensitivity of corneal tissues to chemicals. Nevertheless, vision loss can have serious implications for public health. While UV exposure has its benefits, it can also pose significant risks to eye tissues. Acute and chronic exposure has different detrimental effects on eye health. Although protective measures are essential, chronic exposure to UV radiation for outdoor workers may lead to visual disorders like photokeratitis. Therefore, there is an urgent need for emerging therapies to address UV exposure-related ocular diseases. miRNA-based therapeutics have been instilling hope, yielding successful outcomes in nearly all diseases. Depending on their transcriptional pathway effects, these therapies may pave the way for future pharmacotherapy. miR-330-3p, known for its anti-inflammatory and anti-apoptotic properties, presents a promising treatment option for corneal damage arising from photokeratitis. Our study is the first to explore the alleviating effects of miR-330-3p in photokeratitis, yielding encouraging results. Future studies are suggested to fully understand the underlying mechanisms in acute and chronic high exposure to UV radiation in ocular degenerative disorders.

Ethics

Ethics Committee Approval: Çanakkale University Experimental Research Application and Research Center, with ethical approval from Çanakkale University Animal Experiments Local Ethics Committee (decision no: 2024/04-02, date: 18.04.2024).

Informed Consent: It is an animal experiment study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.A.E., C.A., D.A., Concept: H.A.E., H.E., Design: H.A.E., H.E., Data Collection or Processing: C.A., D.A., Analysis or Interpretation: B.B., Writing: H.A.E., H.E., C.A.

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