Research Article

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Exposure To Subthreshold Dose Of Uvr-B Does Not Induce Increase In Caspase 3 Mrna Expression In The Rat Lens In Vivo Within First 24 Hours.

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ABSTRACT

Purposes: The aim of this study is to investigate the time evolution of caspase 3 mRNA expression within first 24 hours in the rat lens after in vivo exposure to subthreshold dose of UVR-B.

Methods: Twelve six-week-old female albino Sprague-Dawley rats were exposed to subthreshold dose (1 kJ/m2) of UVR-B unilaterally and sacrificed at 1, 8, 16 and 24 hours after exposure. Lenses were enucleated and caspase 3 mRNA expression was detected by qRT-PCR. The time evolution of caspase 3 mRNA expression was then plotted as a function of mean ratios in caspase 3 mRNA expression between exposed and contralateral nonexposed lenses.

Results: There is no difference in mean ratios in caspase 3 mRNA expression between exposed and nonexposed lenses in all four postexposure groups.

Conclusions: Exposure to subthreshold dose of UVR-B does not induce increase in caspase 3 mRNA expression in the rat lens in vivo within first 24 hours.

Translational relevance: Preclinical animal model study of ultraviolet radiation B cataract might lead to the development of new non-surgical therapy of cataract.

Keywords : Lens; Uvr-B; Caspase 3; Time Evolution; Cataract; In Vivo; Qrt-Pcr.

INTRODUCTION

Ultraviolet radiation is a radiation in range of electromagnetic spectrum between 100 nm and 400 nm. It is divided on ultraviolet radiation A (315-400 nm), ultraviolet radiation B (280-315 nm) and ultraviolet radiation C (100-280 nm) [1]. The most mutagenic ultraviolet radiation is the ultraviolet radiation C. It causes mutations that can be carcinogenic though solar ultraviolet radiation C does not reach the Earth surface due to its absorption in the atmosphere. Ultraviolet radiation B is the radiation that is responsible for damage in the lens and skin. Ultraviolet radiation B causes direct DNA damage by producing pyrimidine dimers. Pyrimidine dimers are repaired by nucleotide excision repair. If pyrimidine dimers do not repair, they lead to apoptosis or mutations [2]. Ultraviolet radiation B (UVR-B) is the risk factor for development of cortical cataract in humans. It has been extensively studied by many epidemiological studies [3–5]. Today the only treatment for cataract is cataract surgery. There is no therapeutic treatment for cataract available on the market. Further treatment strategies are needed to cut the burden of cataract on health care system. Therefore, understanding of molecular mechanisms of cataract is required to find medical treatment for cataract.

Lately in our laboratory we focused on apoptosis in the rat lens after in vivo exposure to UVR-B, particularly on caspase 3. It was found that caspase 3 is expressed both on protein and mRNA levels in the rat lens after in vivo exposure to different doses of UVR-B [6–10]. It was found that there is an mRNA expression of caspase 3 in the rat lens at 120 hours after in vivo exposure to UVR-B 6,8. So, to understand the time evolution of caspase 3 in the rat lens after in vivo exposure to subthreshold dose of 1 kJ/m2 of UVR-B this study was designed. qRT-PCR was used as the method for investigation. So, the purpose of the study is to investigate the time evolution of caspase 3 mRNA expression within first 24 hours in the rat lens after in vivo exposure to subthreshold dose of UVR-B.

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MATERIALS AND METHODS

Animals

Six-week-old albino Sprague-Dawley (SD) female rat (Taconic, Denmark) was the experimental animal. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. The Uppsala Ethics Committee on Animal Experiments has given the ethical approval with the protocol number 5.2.18-8927/16.

Exposure to ultraviolet radiation

UVR source

The high-pressure mercury arc lamp (model 6828; Oriel, Stratford CT) generated UVR-B at 300 nm (UVR-300 nm). The radiation was collimated, passed through a water filter, and focused on the entrance slit of a double monochromator. The given radiation had a spectral distribution centered at 300 nm [11]. The thermopile (model 7101; Oriel, Stratford CT) calibrated to a US National Institute of Standard traceable source measured the intensity of UVR.

UVR exposure

A mixture of 90 mg/kg ketamine and 10 mg/kg xylazine was injected intraperitoneally to anesthetize the animal fifteen minutes before the exposure. After that, the rat was placed in a rat holder [12], and tropicamide 10 mg/ml was given in both eyes to induce mydriasis. A subthreshold dose of 1 kJ/m2 of UVR-300 nm during 15 minutes [13] was given to one eye of each animal, while the other eye was covered during the exposure [14].

After a certain postexposure period, the rat was sacrificed by carbon dioxide asphyxiation. The eyes were removed, and the lenses were extracted. Remnants of the ciliary body were removed from the lens equator, keeping the lens in balanced salt solution (BSS; Alcon, USA).

qRT-PCR

NucleoSpin RNA II (Macherey-Nagel GmbH & Co, Duren, Germany) was the RNA isolation kit. The RNA concentration in the analyte was measured as absorbance in a NanoDrop ND-1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA), and the cDNA was synthetized using 1 µg of total RNA by 1st strand cDNA synthesis kit, Roche Diagnostics GmbH, Mannheim, Germany.

The cDNA made from lens RNA was analyzed by quantitative real-time PCR on a 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA USA). The TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA, USA) was used with the TaqMan assays for caspase 3 (Rn00563902_m1) and 18s (Hs99999901_s1), according to the manufacturer's instructions. Primary fluorescence measurements were fitted with the 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) algorithm and threshold fluorescence was selected standardized for each PCR plate by the algorithm. The number of cycles at threshold fluorescence was used as the measurement in a 7500 Fast Real-Time PCR System software.

Experimental Design

Altogether 12 rats were used in the experiment. Each animal was exposed in vivo to UVR-300 nm in one eye. Animals were sacrificed at 1, 8, 16 and 24 hours after exposure to UVR-B, three animals in each group. Samples from all lenses were processed for qRT-PCR of caspase 3 mRNA. 18s was used as a reference gene for each lens sample. Firstly, ratios between Ct values of caspase 3 and 18s were calculated. Further, ratios between exposed and nonexposed lenses were found.

Statistical parameters

The significance level and the confidence coefficient were set to 0.05 and 0.95, respectively, considering sample size.

RESULTS

It was found that there was no difference in mean ratios in caspase 3 mRNA expression between exposed and nonexposed lenses in all four postexposure groups (Figure 1).

Figure 1.

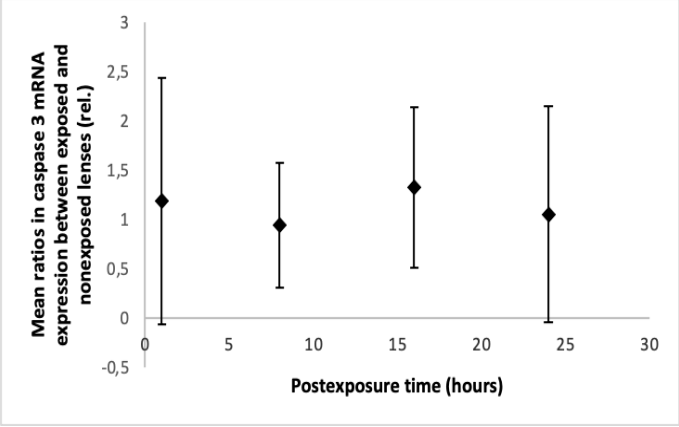

Figure 1. Evolution of caspase 3 mRNA expression in the rat lens after in vivo exposure to 1 kJ/m2 of UVR-B. Error bars are 95% confidence intervals for mean ratios in caspase 3 mRNA expression between exposed and nonexposed lenses.

DISCUSSION

The study was designed to investigate the time evolution of caspase 3 mRNA expression during the first 24 hours in the rat lens after in vivo exposure to subthreshold dose of UVR-B. The albino Sprague-Dawley female rats, in contrast to males, were chosen based on the fact that they have less allergic

urine. Moreover, there is no difference in gender in relation to severity of UVR induced cataract [15].

The reason to dilate pupil in both eyes of the rats was to reduce the variation in pupil size during the exposure to UVR-B.

The dose 1 kJ/m2 of UVR at 300 nm was selected to be a subthreshold dose [16] that induces caspase 3 mRNA expression in the rat lens in vivo [7, 9].

The time intervals of 1, 8, 16 and 24 hours after the exposure were chosen based on previous investigation [10] that finds a peak of active caspase 3 in the rat lens at 16 hours after in vivo exposure to UVR-B at 300 nm.

The finding that caspase 3 mRNA expression is not increased in the rat lens in all four postexposure groups indicates that it might take longer time than 24 hours for onset of caspase 3 mRNA expression in the rat lens after in vivo exposure to subthreshold dose of UVR-B. This is supported by our recent results that exposure to subthreshold dose of 1 kJ/m2 of UVR-B leads to increased expression of caspase 3 mRNA in the rat lens in vivo at 120 hours [7, 9]. The delay in onset of expression of caspase 3 mRNA after exposure to subthreshold dose of UVR-B could be explained by the mechanism of photochemical reaction where the effect occurs with the delay after stimulus. Another understanding could lie in an explanation that transcription machinery for caspase 3 in the rat lens is not triggered by subthreshold dose of UVR-B within first 24 hours and it takes a higher dose of UVR-B to start the expression of caspase 3 mRNA. On the contrary, the transcription of caspase 3 mRNA starts with initial downregulation found in the rat lens exposed to 8 kJ/m2 of UVR-B [8]. Further research is needed to study the time evolution of caspase 3 mRNA expression in the rat lens after 24 hours postexposure.

Caspase 3 participates in the apoptosis pathway as an executor. It activates the endonuclease CAD that leads to DNA degradation [17]. Interestingly, transcription of caspase 3 mRNA is the first step in the process of activation the caspase 3 leading to translation of caspase 3 protein. Further, posttranslational modifications are required to activate the caspase 3. Here, in this study we present the transcription of caspase 3 mRNA in the rat lens after in vivo exposure to subthreshold dose of UVR-B. Further studies are required to understand the translation and posttranslational modifications of caspase 3 protein in the rat lens.

In this study it was found that 95% confidence intervals for the mean are quite wide in 1 hour and 24 hour groups. One explanation to this finding could be the high variation in caspase 3 mRNA expression in these groups. This could be caused by small sample size of three animals in the group. On the contrary, groups of 8 hours and 16 hours have quite normal 95% confidence intervals for the mean with the same sample size as in 1 hour and 24 hour groups. Another probable explanation to this is that transcription machinery for caspase 3 mRNA in groups 1 hour and 24 hours is less sensitive to UVR-B than in groups 8 hours and 16 hours meaning that subthreshold dose of UVR-B does not produce the same strong caspase 3 mRNA expression in groups 1 hour and 24 hours as in groups 8 hours and 16 hours. Further, it takes longer time for 24 hour group than other groups to respond to UVR-B stimuli and it is a high stress for 1 hour group to respond to UVR-B stimuli. Further research with larger sample size is needed to verify this hypothesis.

CONCLUSION

In conclusion, exposure to subthreshold dose of UVR-B does not induce increase in caspase 3 mRNA expression in the rat lens in vivo within first 24 hours.

Acknowledgements

Ethics approval

All methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Ethical approval was obtained from the Uppsala Ethics Committee on Animal Experiments, protocol number 5.2.18-8927/16.

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Competing interests

All authors do not have a conflict of interest to declare.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contribution

All authors designed, performed the experiment, analyzed the data and wrote and revised the manuscript.

Consent for publication

Not applicable.

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