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Research Article

Exposure to Subthreshold Dose of UVR-B Does Not Induce Apoptosis in the Rat Lens *In-vivo* within First 24 hours

Konstantin Galichanin*, Lovisa Falkman, Zhaohua Yu

Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

ABSTRACT

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

Methods: Twenty-six-week-old female albino Sprague-Dawley rats were exposed to subthreshold dose (1 kJ/m^2) of UVR-B unilaterally and sacrificed at 1, 8, 16 and 24 hours after exposure. Lenses were enucleated and active caspase 3 was detected by western blot. The time evolution of active caspase 3 was then plotted as a function of relative mean difference in active caspase 3 between exposed and nonexposed lenses.

Results: There is expression of active caspase 3 in both exposed and nonexposed lenses but there is no difference in relative mean difference in active caspase 3 between exposed and nonexposed lenses in all four postexposure groups.

Conclusions: Exposure to subthreshold dose of UVR-B does not induce apoptosis in the rat lens *in vivo* within first 24 hours. Apoptosis occurs in the rat lens *in vivo* independently from the exposure to subthreshold dose of UVR-B.

Keywords: Lens; UVR-B; Active caspase 3; Time evolution; Cataract; *In vivo*; Western blot

INTRODUCTION

The aim of this study is to investigate the time evolution of active caspase 3 during the first 24 hours in the rat lens after *in vivo* exposure to subthreshold dose of UVR-B.

There have been research studies done on evolution of apoptosis in the rat lens *in vivo* after exposure to different doses of UVR-B [1-8]. Those studies included several molecular biological techniques such as light microscopy, transmission electron microscopy, tunel staining, qRT-PCR, immunohistochemistry and western blot [2-8]. Light and transmission electron microscopy identified typical apoptotic features. Tunel staining visualized tunel-positive staining. qRT-PCR studied mRNA expression of TP53 and CASP3. Immunohistochemistry and western blot analyzed expression of p53 and active caspase 3 proteins.

Previous study based on tunel staining by Michael, et al., [1] finds that there is a peak of tunel-positive staining in the rat lens *in vivo* at 24 hours after exposure to close to threshold dose (5 kJ/m^2) of UVR-B. Another study on tunel-staining by Kronschlager, et al., [5] shows that there is a peak of tunel-positive staining in the rat lens *in vivo* in the time interval of 5-120 hours after exposure to close to threshold dose (5 kJ/m^2) of UVR-B. Further, a study based on immunohistochemistry of active caspase 3 shows that there is a peak of active caspase 3 expression in the rat lens *in vivo* at 16 hours after exposure to subthreshold dose (1 kJ/m^2) of UVR-B. Another study based on immunohistochemistry of active caspase 3 reveals that there is a positive staining of active caspase 3 at 7 days after exposure to double threshold dose (8 kJ/m2) of UVR-B [2]. Four studies on qRT-PCR reveal that exposure to double threshold dose (8 kJ/m^2) induces increased mRNA expression of caspase 3 in the rat lens *in vivo* at both 120 hours and 7 days and exposure to subthreshold dose (1 kJ/m^2) of UVR-B results in increased mRNA expression of caspase 3 in the rat lens *in vivo* at 120 hours. Further, a study on light and transmission electron microscopy by Galichanin, et al., [3] indicates microscopic features of apoptosis already at 1 hour after exposure to double threshold dose (8 kJ/m^2) of UVR-B. And a study on western blot by Galichanin [8] shows an active caspase 3 in the lens epithelial cells at 120 hours after exposure to subthreshold dose (1 kJ/m^2) of UVR-B.

Correspondence to: Konstantin Galichanin, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden; E-mail: konstantin.galichanin@neuro.uu.se

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It seems that there is an early time delay within 24 hours and a late time delay within 120 hours and 7 days of onset of apoptosis in the rat lens after exposure to UVR-B. Lately, our research group focused on active caspase 3 protein expression in the rat lens *in vivo* after exposure to subthreshold dose of UVR-B. Active caspase 3 was chosen based on its crucial role in execution of apoptosis and therefore was used as a marker for apoptosis. To clarify the onset of apoptosis, the current experiment on active caspase 3 protein was designed. The western blot technique was used in the study. So, the purpose of the study is to investigate the time evolution of active caspase 3 within first 24 hours in the rat lens after *in vivo* exposure to subthreshold dose of UVR-B.

MATERIALS AND METHODS

Animals

The experimental animal was the six-week-old albino Sprague-Dawley (SD) female rat (Taconic, Denmark). 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.

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 emerging radiation had a spectral distribution centered at 300 nm with dual peaks at 297.5 nm and 302.6 nm with 10.2 nm full width at half maximum [3]. 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. Thereafter, the animal was placed in a rat holder and tropicamide 10 mg/ml was instilled in both eyes to induce mydriasis [9]. A subthreshold dose of 1 kJ/m^2 of UVR-300 nm during 15 minutes was applied to one eye of each animal, while the contralateral eye was shielded during the exposure [10,11].

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

Western blot

The lens samples were separated on a mini-protean TGX stain-free (12% gel) (Bio-rad, Hercules, CA, USA), transferred onto nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) and incubated sequentially with primary and appropriate horseradish peroxidase conjugated secondary antibodies. Primary antibodies were rabbit polyclonal cleaved caspase-3 antibody Asp175 (9661; cell signalling technology, Inc., Danvers, MA, USA) for caspase 3 protein and mouse monoclonal anti-β-actin antibody (ab8227; Cambridge, UK) for β-actin protein that served as a reference protein. Secondary antibody was polyclonal peroxidase goat anti-rabbit HRP-linked IgG (7074S; cell signalling technology, Inc., Danvers, MA, USA). Signals were detected using the Clarity Western ECL reagent kit (Bio-Rad, Hercules, CA, USA). Pictures were obtained by the imaging system Li-Cor Odyssey FC (LI-COR Biosciences, Lincoln, NE, USA) and analyzed with image J software.

Experimental design

Altogether 20 rats were used in the experiment. One eye in each animal was exposed *in vivo* to UVR-300 nm. Animals were sacrificed at 1, 8, 16 and 24 hours after exposure to UVR-B, five animals in each group. Samples from all lenses were processed for Western blot of active caspase 3 protein. β-actin was used as a reference protein for each lens sample. Firstly, ratios between number of pixels of active caspase 3 and β-actin was calculated. Secondly, ratios between exposed and nonexposed lenses was found. Finally, relative mean difference in active caspase 3 between exposed and nonexposed lenses was calculated.

Statistical parameters

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

RESULTS

There is an expression of active caspase 3 for both exposed and nonexposed lenses in all four postexposure groups. It was found that there was no difference in relative mean difference in active caspase 3 between exposed and nonexposed lenses in all four postexposure groups (Figure 1).

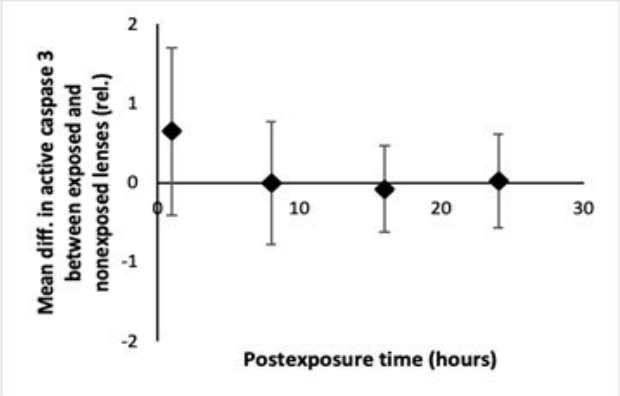

Figure 1: Evolution of mean difference in active caspase 3 in the rat lens after *in vivo* exposure to 1 kJ/m^2 of UVR-B. Error bars are 95% confidence intervals for mean difference between exposed and non-exposed lenses.

DISCUSSION

The study was designed to investigate the time evolution of active caspase 3 during the first 24 hours in the rat lens after *in*

vivo exposure to subthreshold dose of UVR-B. The dose 1 kJ/m^2 of UVR at 300 nm was selected to be a subthreshold dose that induces expression of active caspase 3 in the rat lens *in vivo* [12]. The time intervals of 1, 8, 16 and 24 hours after the exposure were chosen based on previous investigation 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 current finding that there is an expression of active caspase 3 for both exposed and nonexposed lenses in all four postexposure groups is in concordance with the results of Talebizadeh N, et al., [13] that finds an expression of active caspase 3 in normal rat lenses. Authors show that there is a higher expression of active caspase 3 in the central zone of the lens than in the equatorial zone and discuss higher protease capacity to be an explanation for the difference in expression of active caspase 3 in opposed regions of the lens. Zandy A, et al., [14] discuss in their research work of the role of caspase 3 in the normal mouse lens and finds that caspase 3 is not required for organelle loss in the lens and caspase 3 might contribute to the lens transparency. Our finding shows that expression of active caspase 3 occurs in the rat lens independently of the exposure to UVR-B suggesting normal protein turnover in the rat lens.

Our observation that there is no relative mean difference in active caspase 3 between exposed and nonexposed lenses in all four postexposure intervals suggests that apoptosis does not occur within first 24 hours followed by the exposure to subthreshold dose of UVR-B. The probable explanation to this finding could be that apoptosis induced by subthreshold dose of UVR-B requires more time to develop and might occur at later time intervals after exposure to subthreshold dose of UVR-B. The previous findings confirm that exposure to subthreshold dose of UVR-B results in higher expression of active caspase 3 on mRNA and protein levels in exposed lenses compared to nonexposed lenses in later time interval as 120 hours. Another observation indicates that exposure to double threshold dose of 8 kJ/m^2 UVR-B induces mRNA and protein expressions of active caspase 3 in rat lenses at 7 days. These studies might suggest that UVR-B induced apoptosis in the rat lens conforms a phenomenon of photochemical effect of UVR-B on the lens where the onset of an event occurs with a time delay. The studies on lens light scattering measurements after exposure to UVR-B support this phenomenon [15]. Further research is required to confirm the role of a photochemical reaction in UVR-B induced apoptosis in the rat lens.

CONCLUSION

In conclusion, exposure to subthreshold dose of UVR-B does not induce apoptosis in the rat lens *in vivo* within first 24 hours.

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 analyzed 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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