Oxidized mitochondrial flavins/NAD(P)H ratio and 8-oxo-7,8-dihydroguanine formation in cornea after ultraviolet-A irradiation

Corinne Zinflou and Patrick J. Rochette

Axe Médecine Ré管家tique, Centre de Recherche du CHU de Québec - Université Laval, Québec, Canada.
Faculté de médecine, Université Laval, Québec, Canada.

INTRODUCTION

- Prolonged exposure to sunlight ultraviolet (UV) irradiation is toxic for the eye.
- UV A wavelength (315-400 nm), the main component of solar UV reaching the eye, is the most penetrating in tissues and cause oxidative stress.
- UV A enhance reactive oxygen species (ROS) production and ROS-mediated oxidative DNA damage, mostly 8-oxo-7,8-dihydroguanine (8-oxodG).
- 8-oxodG has been linked to numerous ocular diseases, such as age-related macular degeneration, cataracts, faults endothelial corneal dystrophies, keratoconus and pterygium.

RESULTS

As the anterior structures of rabbit eyes have wavelength filtering capacities similar to those of human eyes, anterior structures were used to assess the consequences of UVA exposure in cornea (Fig. 3). Two markers were used to investigate oxidative stress: oxidized mitochondrial flavins (mitochondrial NAD(P)H ratio) as a marker of mitochondrial activity and redox state (Fig. 4), and 8-oxodG as a marker of photo-oxidative DNA damage.

METHODS

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ROS and oxidative DNA damage are etiologic factors.

Figure 1: Anatomy of the eye depicting the main ocular degenerative diseases in which UV A-induced oxidative stress might trigger mitochondrial anomalies in those parts. Such a disturbance, favored by a chronic UV A exposure on quiescent endothelial cells, for example, could lead to long-term cellular dysfunction and increased apoptosis, observed in faults endothelial corneal dystrophies.

Figure 2: Anatomy of the rabbit cornea

To investigate the extent of UV A-induced oxidative stress and its implication in rabbit cornea (Fig. 2), following an exposure of the eyes to UV A-light.

AIM

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Figure 3: Experimental images. Rabbit eyes were used to assess the consequences of UV A exposure in cornea. The right panel: one eye was irradiated with UVA (lower panels) and, while the other side was not irradiated. The cornea was removed immediately after irradiation. Using the microscope (Zeiss Axiovert 200M), the anterior structures were observed with a 10x objective. Mitochondria were counterstained with Mitotracker (red) to show mitochondria in the deepest corneal parts, posterior stroma and endothelial sides.

Figure 4: 8-oxodG/NADPH ratio analysis. B) Immunofluorescence signals within cornea from rabbit eyes irradiated with 6000 kJ/m² UVA (lower panel), non-irradiated eyes (upper central panel). Oxidized mitochondrial flavins (green) and reduced NADPH (red) were evaluated using the microscope excitation spectra of each coenzymes. Mitochondria were counterstained with Mitotracker (red) to show mitochondria in the deepest corneal parts, posterior stroma and endothelial sides.

Figure 5: Oxidized and reduced forms of mitochondrial flavins (NAD(P)H and Fv) and mitochondrial and nuclear DNA oxidodG analysis in cornea following UV A exposure. Oxidized mitochondrial flavins (red) and mitochondrial DNA (top panels) and reduced mitochondrial flavins (green) and nuclear DNA (middle panels) in control and UV A-irradiated rabbit corneas were used as negative controls (upper panels) Oxidized mitochondrial flavins fluorescence (green) and reduced NAD(P) fluorescence intensity measurements within cornea (left panels) and UVA (lower panels). Unirradiated rabbit eyes, and estimated the 8-oxodG/A irradiation.

PERSPECTIVES

- Monitor the UV A/NADPH ratio in endothelia at different times post-irradiation to assess whether UV A-induced mitochondrial metabolic disruption is reversible or not.
- Investigate the extent of UV A-induced oxidative stress and its consequences.

CONCLUSIONS

- UV A toxicity in the cornea is not only the result of oxidative DNA damage, but also of mitochondrial metabolic disruption.
- UV A/NADPH ratio analysis and 8-oxodG distribution suggest that mitochondria in the deepest corneal parts (posterior stroma and endothelium) are more sensitive to photo-oxidative stress.
- UV A-induced oxidative stress might trigger mitochondrial anomalies in those parts. Such a disturbance, favored by a chronic UV A exposure on quiescent endothelial cells, for example, could lead to long-term cellular dysfunction and increased apoptosis, observed in faults endothelial corneal dystrophies.

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REFERENCES