Time-dependence of UVB-induced cellular mechanisms in cultured human keratinocytes

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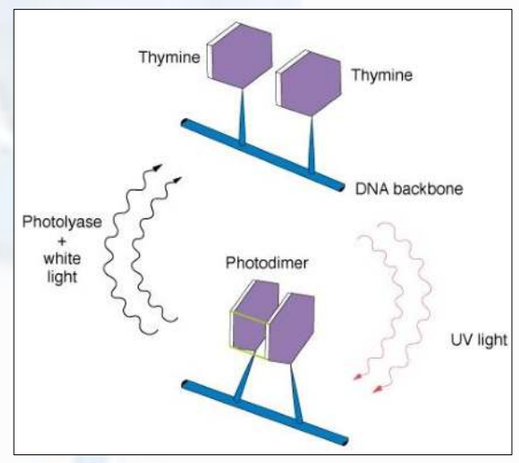
UVB-induced cyclobutane pyrimidine dimers (CPDs) are considered to be the main cause of acute sunburn and epidermal carcinogenesis. In humans, these lesions are repaired by nucleotide excision repair (NER), but marsupials and lower organisms present a more effective **photolyase enzyme** which rapidly removes CPDs in a visible light-dependent process (photoreactivation).



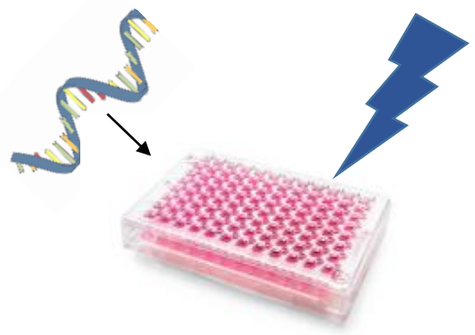
According to the clinical experiences, there is a **need for treatment to diminish the effects of sunburn 6-8 hours after UVB** exposure when the first symptoms appear. Nonetheless, there is no approved therapy to effectively reduce the damage.

Previously we have established a method in which human keratinocyte cell lines were transfected with **pseudouridine-modified mRNA encoding CPD-specific photolyase** [1], and this approach was suitable to avoid UVB-induced apoptosis and to determine **CPD-dependent processes in time-course studies**.

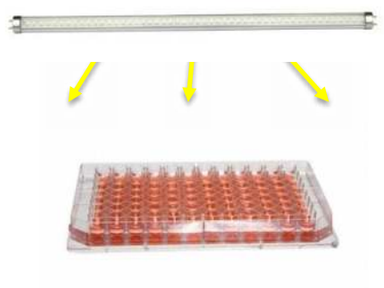
Aim of the study: **How much time do we have to reduce UVB-induced keratinocyte damage after exposure?**

Materials and methods

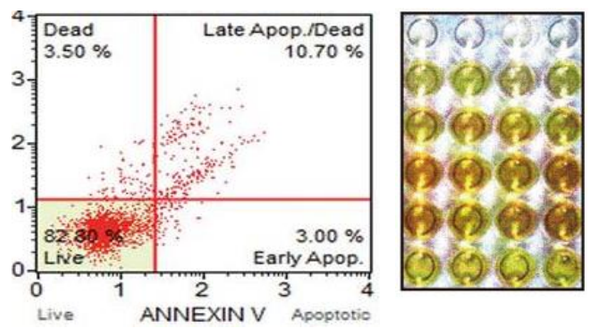
In vitro cell cultures: HaCaT; NHEK



Lipofectamine-complexed mRNA transfection followed by 60 mJ/cm^2 UVB irradiation

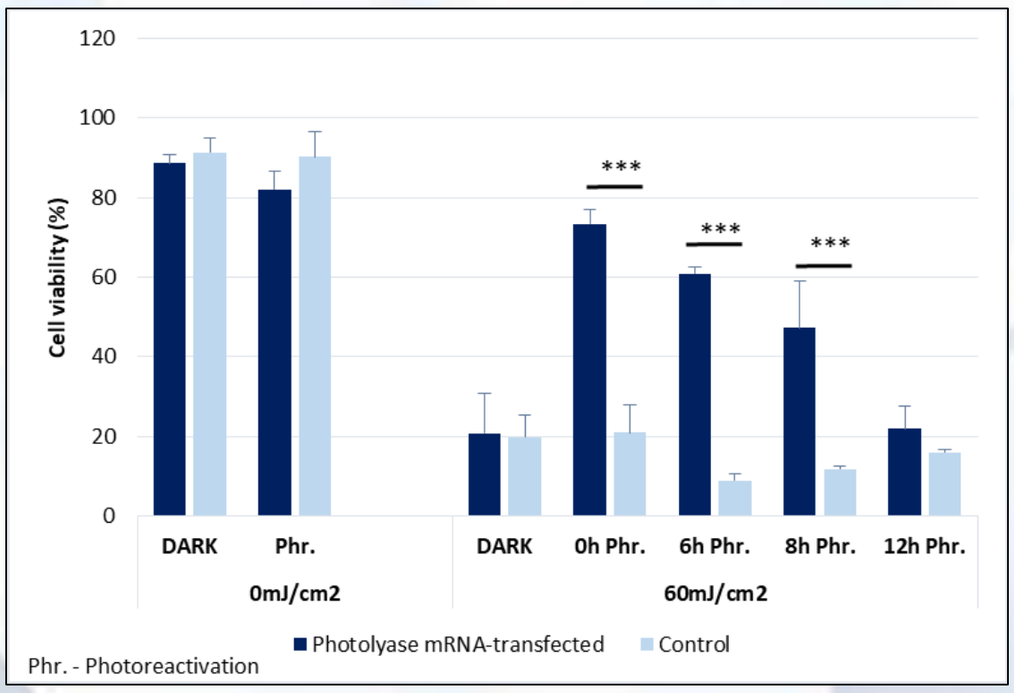


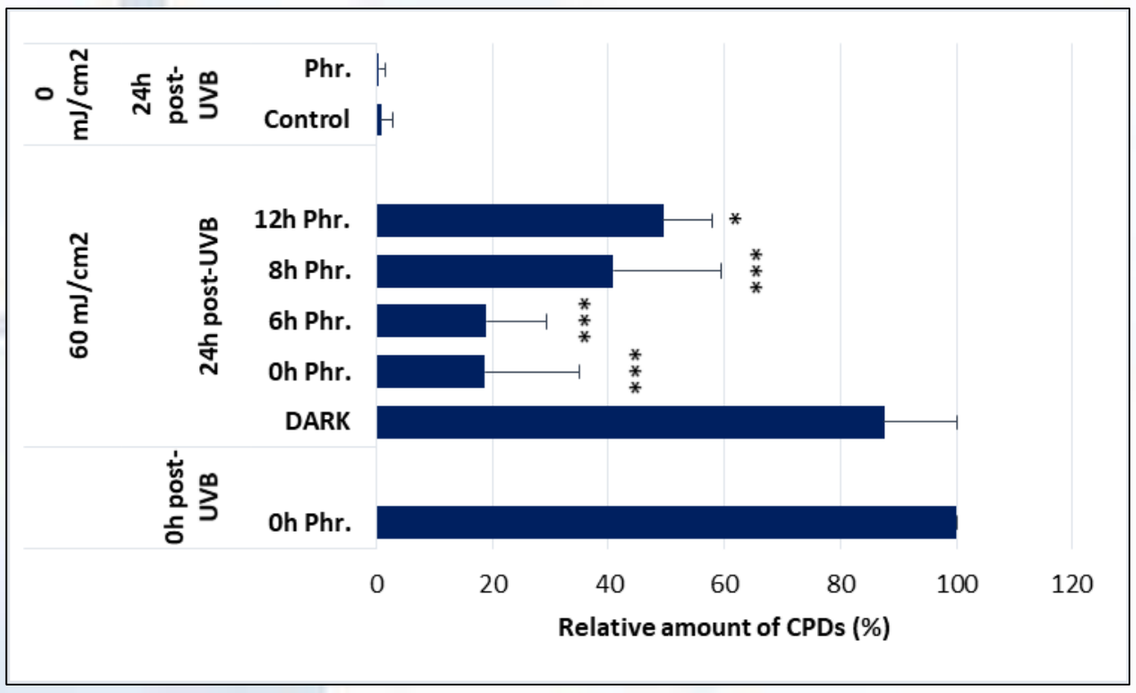
Photoreactivation with visible light 0, 6, 8 or 12 hours after the UVB exposure



Annexin V/PI dual staining followed by flow cytometry; CPD-specific ELISA

Results





Photolyase-mediated CPD-removal restored cell viability close to the baseline conditions up to 6 hours after UVB treatment. Intervention in keratinocyte apoptosis at 8 hours after the UVB injury is still feasible. In comparison with cells in which the photolyase enzyme was not "switched on" the CPD level remained high and rates of cell viability reduced significantly.

In photolyase mRNA-transfected cells 80% of CPDs were removed by CPD-photolyase activated by visible light at 0 and 6 hours post-irradiation. At 8-12 hours after UVB exposure, CPD repair by photoreactivation of photolyase still caused significant decrease in the amount of detectable photolesions.

Conclusion

Our results suggest that keratinocyte apoptosis induced by UV can be prevented within 6-8 hours after the UVB injury by the elimination of CPD photolesions. The activation of the photolyase enzyme at later time point, might not prevent apoptosis.

References

1. Boros G, Miko E, Muramatsu H, Weissman D, Emri E, Rózsa D, Nagy G, Juhász A, Juhász I, van der Horst G, Horkay I, Remenyik É, Karikó K, Emri G. **Transfection of pseudouridine-modified mRNA encoding CPD-photolyase leads to repair of DNA damage in human keratinocytes: a new approach with future therapeutic potential.** J Photochem Photobiol B. 2013 Dec 5;129:93-9.

Acknowledgments

Our work was supported by the European Regional Development Fund co-financed by the European Union (GINOP- 2.3.2-15-2016-00005) and the Hungarian National Research Development and Innovation Fund (OTKA K120206).