Photoprotective properties of myconoside, isolated from *Haberlea rhodopensis*, under UVA/UVB-induced cytotoxicity in HaCaT cells

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Introduction

The skin is the main organ to protect human body from the harmful effects of the environmental stimuli, such as chemicals and ultraviolet (UV) radiation. Solar UV light is the most common hazard associated with skin impairment, causing oxidative stress, skin burns, inflammation, erythema and breakdown of the extracellular matrix. Extensive UV exposure may induce photoaging and skin cancer (Li et al., 2020; Natarajan et al., 2014). Research and development of effective, ecological safety, non-toxic topical sunscreens remains a challenge for the pharmaceutical and cosmetics industries, despite growing knowledge of mechanisms of photoaging. Natural photoprotective substances (secondary plant metabolites biosynthesized in response to solar simulated UV radiation) with high photostability and low phototoxicity are able to absorb UVA/UVB radiation, decreasing free radicals and loss of endogenous antioxidants, reducing UV-induced cytotoxicity in primary human epidermal keratinocytes and immortalized human keratinocytes (Kostyuk et al., 2018), but their photoprotective potential has not yet been elucidated.

Aim

Here, we aim to investigate the potential effect of *H. rhodopensis* extract (HRE) and its constituents CAL and MYC on exposure of UVA/UVB radiation.

Materials and methods

Cell culture

An immortalized human keratinocyte (HaCaT) cell line was obtained from Cell Line Service GmbH (Eppelheim, Germany). Cells were cultured following the previously described conditions (Koycheva et al., 2021).

*Haberlea rhodopensis* Friv. (family Gesneriaceae), commonly known as Rhodope silvriak or Orpheus’ flower, is a resurrection plant species endemic for Bulgaria, with therapeutic potential due their antioxidant or redox activity. Its biologically active phenylethanoid glycosides calceolarioside E (CAL) and myconoside (MYC) might regulate the cellular redox homeostasis oxidative stress-associated pathologies (Amirova et al., 2021), but their photoprotective potential has not yet been elucidated.

UV source, dosimetry and irradiation

BS02 UV/VIS irradiation chamber (#860902) purchased from Opsytec Dr. Grobel. GmbH (Ettlingen, Germany), equipped with UVA and UVB lamps and UV-MAT irradiation controller (#820920) was used to irradiate HaCaT (in PBS), and non-irradiated cells were used as a background control. The UV-MAT continuously measures irradiances, calculates doses, and stops irradiation at the set target dose. The two spectral ranges were separately controlled, ensuring a constant dose, measuring range: 0–200 mW/cm². The output was
quantified using radiometric sensors for UVA (315–400 nm, peak 352 nm) and for UVB (280–315 nm, peak 312 nm), respectively. To mimic solar simulated UV radiation the lamps’ emission was filtered by UV-MAT and area attenuator, resulting an intensity which equivalent to a spectrum of 95% UVA and 5% UVB.

Keratinocytes were washed in PBS and then were exposed to either UVA (5–20 J/cm²) or UVB (10–100 mJ/cm²) or UVA/UVB ratio (4.75/0.25–19.0/1.0 J/cm²) radiation at a distance of 20 cm from the source. After UV exposure, PBS was discarded and serum-free medium was applied.

Cytotoxicity and phototoxicity of the extract and test compounds

The HaCaT cells (1.5 x 10⁴ cells/well) were seeded in 96-well plates and were cultured for 48 h to reach confluence. Then cells were treated with HRE (1, 5, 10, 20, 50, 100 µg/mL), CAL and MYC both (1, 5, 10, 20, 50, 100 µM) or methanol (0.05%, v/v). On the 24th hour of treatment MTT assay was conducted as previously described by Koycheva et al. (2021).

To determine phototoxicity keratinocytes were pre-treated with the above concentrations of HRE, CAL, MYC and with vehicle alone in DMEM for 1 h. Then cells were exposed to UVA/UVB radiation (4.75 J/cm² for UVA and 0.25 J/cm² for UVB). After 24 h incubation (37 °C, 5% CO₂), cell damage was evaluated by MTT incorporation into viable cells.

Results and discussion

To support HRE, CAL and MYC topical applications, an in vitro dermatological study of cytotoxic, phototoxic and UVA/UVB photoprotective effects using HaCaT was done. The MTT assay, which measures mitochondrial dehydrogenase enzyme activity, was employed to determine the cell viability.

A dose-dependent reduction of HaCaT cell viability up to 32.0% and 2.34% was observed under UVB- and UVA/UVB-induced cytotoxicity, respectively. While the cell viability was not influenced remarkably to UVA radiation, decreasing with 7.82%.

Exposure to different concentrations of HRE, CAL and MYC for 24 h did not affect significantly the cell viability up to 100 µg/mL for the extract and 100 µM for the pure compounds, respectively. The HRE and MYC demonstrated photoprotective potential on UVA/UVB irradiation.

Conclusion

The results obtained could be used for further biology studies on HRE, CAL and MYC and evaluations of their molecular mechanism of action. Furthermore, the UVA/UVB photoprotection of HRE and MYC could also be utilized in the development of new MYC-based products for topical application.

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References


