Protective Effects of Quercetin-Loaded PLGA Nanoparticles against UVA-Induced Photoaging in Mouse Skin

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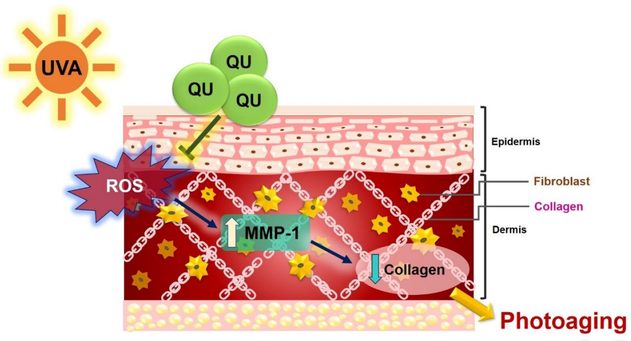
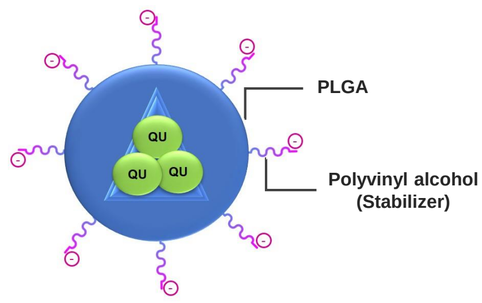
Background

- UVA irradiation induces cellular oxidative stress causing premature aging skin signs such as fine lines and wrinkles.
- Cellular oxidative stress induced by UVA activates the production of metalloproteinase-1 (MMP-1) leading to the degradation of type I collagen in normal human dermal fibroblasts (NHDFs).
- Quercetin (QU), a natural flavonoid, has been shown to reduce damage of photoaging skin in keratinocytes and dermal fibroblasts.
- Poly-lactic-co-glycolic acid (PLGA), a polymer nanoparticle has been approved by USFDA for the use of drug delivery. PLGA - based nanoparticles incorporated with the QU (QU-NPs) were then developed to enhance efficiency of the QU.
- We therefore investigated whether the QU-NPs improve effectiveness of anti-photoaging and alter premature aging parameters.





Methods

- The QU-NPs were synthesized using emulsion-solvent evaporation. Encapsulation efficiency was measured using high performance liquid chromatography (HPLC).
- *In vitro* study, NHDFs were pretreated with QU or QU-NPs 5 and 10 µg/ml for 30 min prior to UVA irradiation of 12 and 24 J/cm^2 for oxidative formation and apoptosis measurements, respectively. The oxidative formation was measured using dichlorofluorescein diacetate (DCFDA) and the number of apoptotic cells was measured using propidium iodide (PI). Fluorescence intensities were determined using flow cytometric analysis.
- *In vivo* study, three-week-old female BALB/c wild type mice were randomized into 8 groups: Group I (sham-control without UVA), Group II (Sham with UVA irradiation), Group III-V (QU 6, 20, 60 µg/ml with UVA) and Group VI-VIII (QU-NPs 6, 20, 60 µg/ml with UVA). The 80% ethanol (sham control), QU and QU-NPs were applied to shaved dorsal skin for 1 h before 10 J/cm^2 UVA irradiation/session, 3 sessions/week for 2 weeks for total cumulative dose of 60 J/cm^2. After euthanization, removed dorsal skin tissues were stained with MMP-1 Ab and collagen I Ab and were assessed by immunofluorescence imaging analysis using ImageJ.

Acknowledgement

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Results

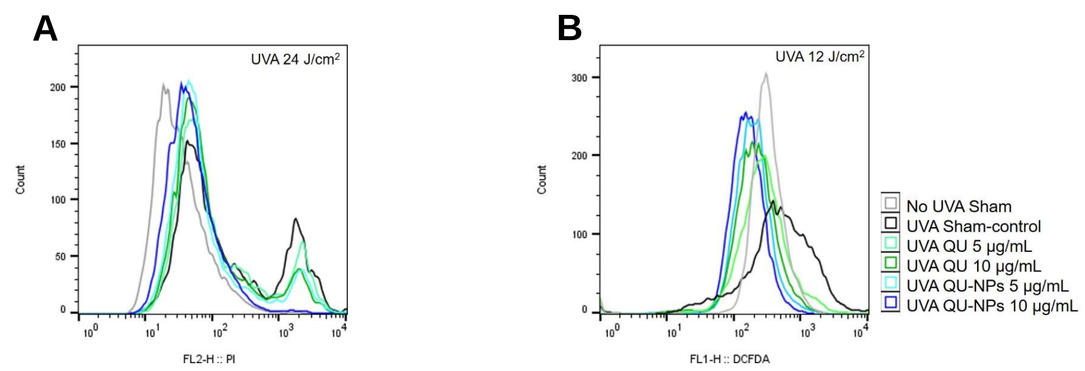

Figure.1 Examples of histogram from an experiment of 30-min pretreated 5 and 10 µg/ml QU or QU-NPs NHDFs prior to UVA irradiation determining the number of apoptotic cells (PI positive cells) (A) and oxidative formation (DCFDA) (B).

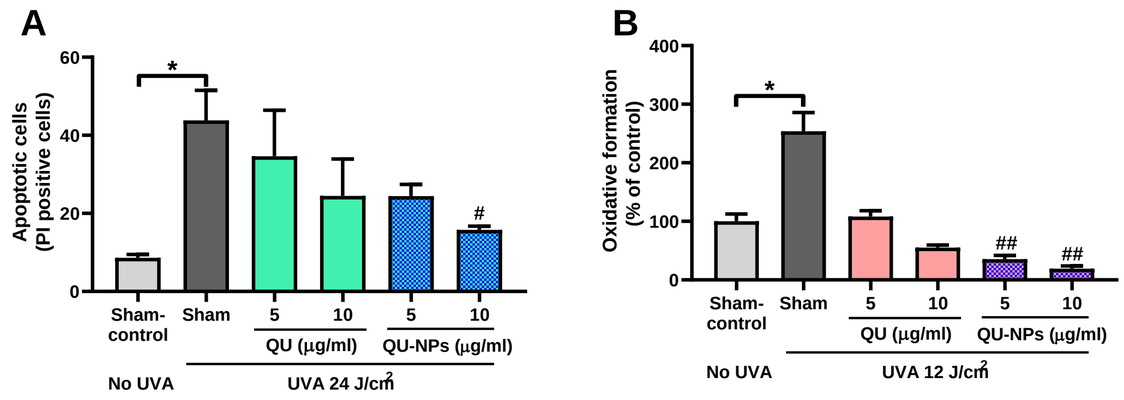

Figure.2 Comparison of apoptotic cells numbers (PI positive cells) of 24-J/cm^2 UVA irradiation of QU or QU-NPs pretreated NHDFs compared to UVA irradiated NHDFs alone (A) and oxidative formation (percentage of control) of 12-J/cm^2 UVA irradiation of QU or QU-NPs pretreated NHDFs compared to UVA treated NHDFs alone (B). **p<0.05 c.f.* the sham-control (no UVA), *#p<0.05; ##p<0.01 c.f.* the sham (UVA)

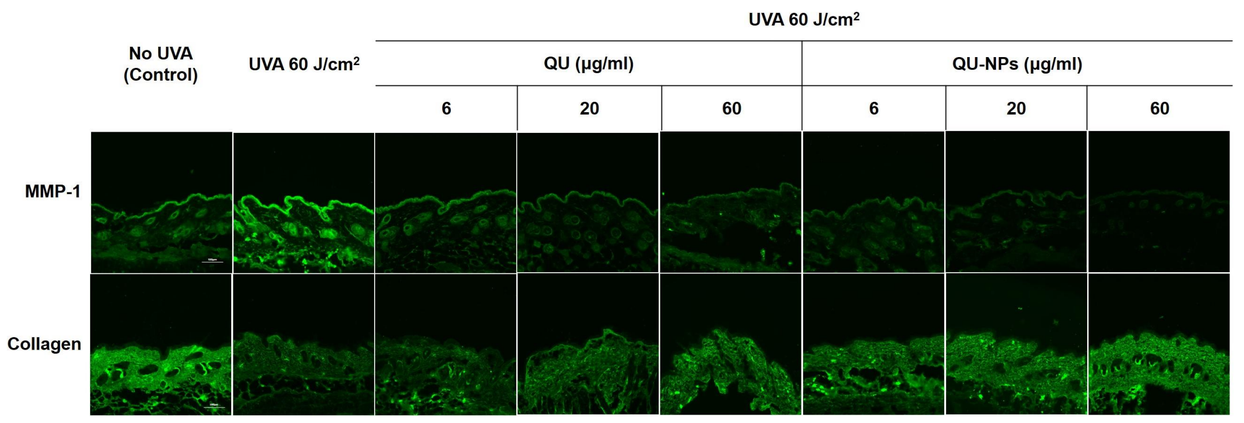

Figure.3 Examples of immunofluorescence images of dorsal skin tissue of mice from an experiment of 1-h pretreated 6, 20, 60 µg/ml QU or QU-NPs NHDFs prior to 10 J/cm^2 UVA irradiation/session, 3 sessions/week for 2 weeks, accumulated 60 J/cm^2 UVA in total. MMP-1 and collagen levels were analyzed using ImageJ.

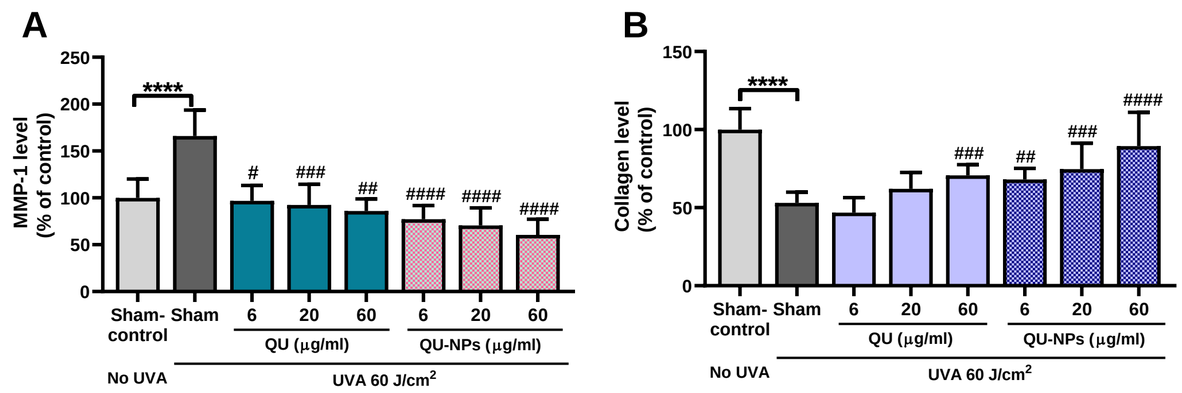

Figure.4 Comparison of MMP-1 (A) and collagen levels (B) of QU or QU-NPs pretreated NHDFs with 60-J/cm^2 cumulative UVA irradiation compared to NHDFs with UVA irradiation alone represented as percentage of control. *****p<0.0001 c.f.* the sham-control (no UVA), *#p<0.05; ##p<0.01; ###p<0.005; ####p<0.001 c.f.* the sham (UVA)

Conclusion

- At the equal amount of total compound mass, less quantity of QU in PLGA nanoparticles compared to free compound significantly protect cytotoxicity and reduce oxidative formation in NHDFs against 24 and 12 J/cm^2 UVA irradiation, respectively. For mouse skin, intermittent topical administration of both QU and QU-PLGA attenuate MMP-1 level while only a maximal QU and all range of QU-PLGA concentrations increase collagen protein level.
- PLGA improved the efficacy of QU in protection against UVA-induced skin photoaging. Thus, the QU-NPs may represent an interesting anti-photoaging candidates.