Phototoxicity induced by chloroquine – an in vitro study using various experimental models

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Background

Chloroquine (CQ) is a medication used to treat and prevent malaria. It is also prescribed for certain autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus. Long-term use of CQ may produce phototoxic side effects both in the skin and in the eye. This may be due to the drug's high affinity for melanin and its accumulation in pigmented tissues. Melanin biopolymers may therefore be of great importance in the development of phototoxicity in patients using chloroquine.

Aim of the study

The purpose of this study was to investigate the phototoxic potential of CQ using various experimental models on skin cells with different pigmentation (human dermal fibroblasts, lightly-pigmented melanocytes, darkly-pigmented melanocytes) and incubation time with the drug prior the exposure to sunlight.

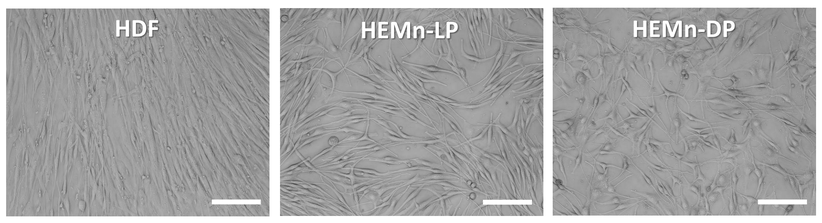
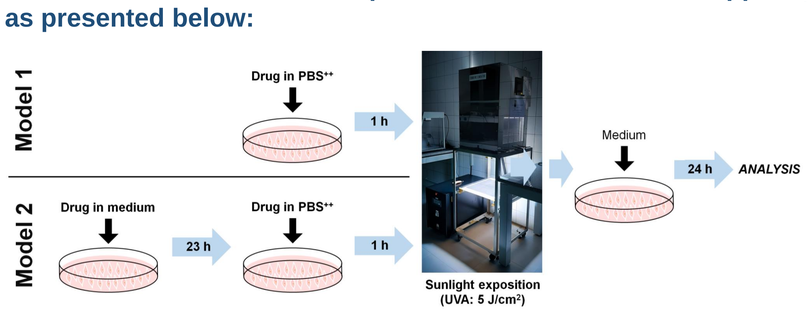


Figure 1. *Representative microsopic images of cell cultures used in the study. The cells were observed under the light inverted microscope NIKON TS100F, scale bar = 50 µm.*

For each cell line two experimental models were applied, as presented below:



Results

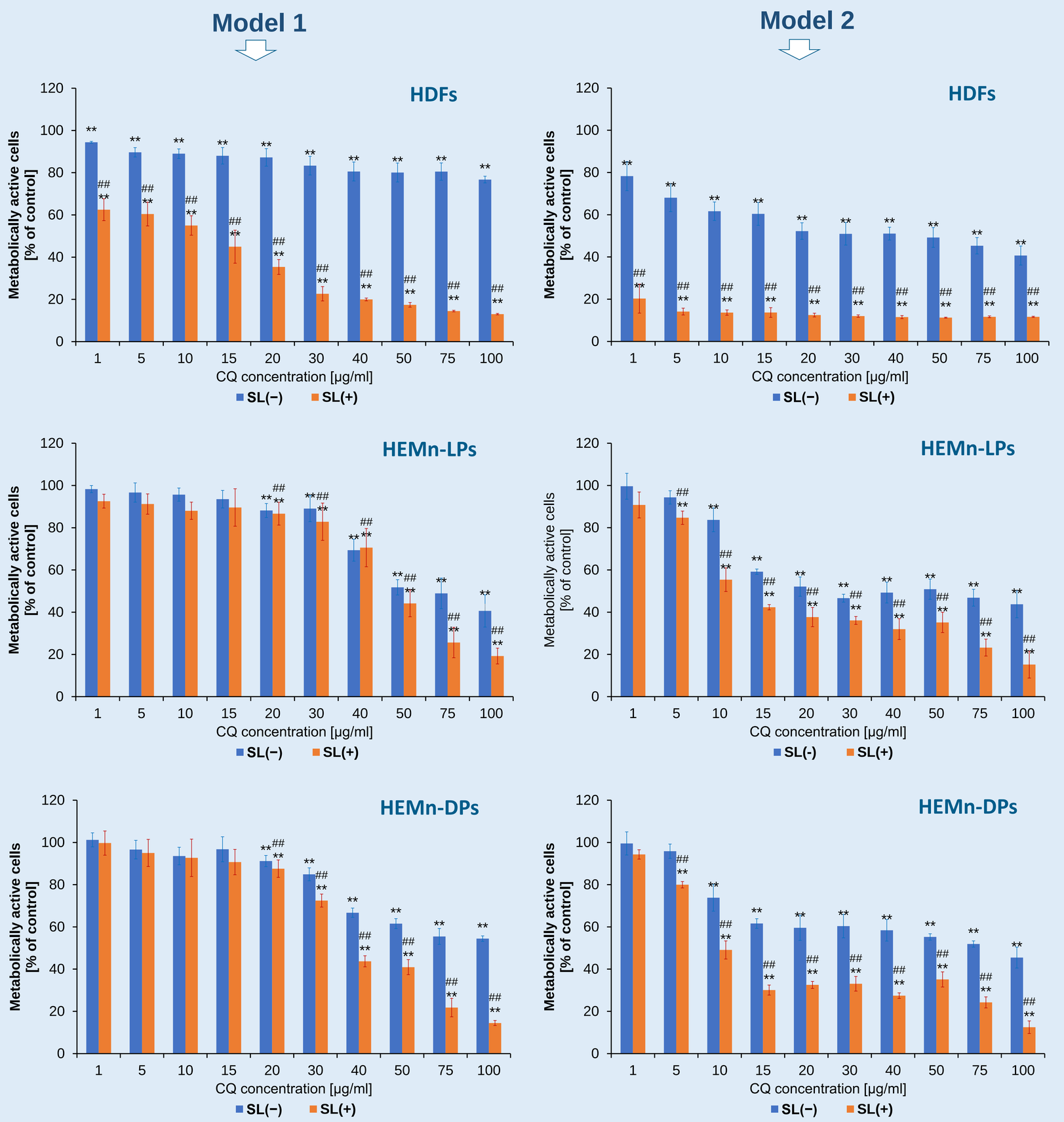


Table 1.
EC_{50} [µg/ml] and PIF (photo-irritation factor) values of chloroquine calculated from WST-1 data.

	Model 1			Model 2		
Cell line	**EC_{50} SL(−)**	**EC_{50} SL(+)**	**PIF**	**EC_{50} SL(−)**	**EC_{50} SL(+)**	**PIF**
HDF	100.0	9.8	10.2	33	0.4	81.3
HEMn-LP	87.9	57.9	1.5	39.9	16.0	2.5
HEMn-DP	109.0	45.8	2.4	51.6	12.6	4.1

**PIF relates the EC_{50} of the viability curve for darkness to the EC_{50} of the viability curve in the presence of sunlight (SL)*

Figure 1.
Effects of chloroquine (CQ) on the metabolical activity of human skin cells non-irradiated or exposed to sunlight (SL). Human dermal fibroblasts (HDFs), human epidermal melanocytes lightly pigmented (HEMn-LPs) and human epidermal melanocytes darkly pigmented (HEMn-DPs) were treated according to experimental model 1 or 2 and tested using the WST-1 assay. The results are demonstrated as the percentage of control, ** $p < 0.005$ vs control, ## $p < 0.005$ vs just irradiated cells.

Methods

Cell culture and treatment

In vitro studies were performed on human dermal fibroblasts (HDF) obtained from Sigma-Aldrich and human epidermal melanocytes (HEMn-LP and HEMn-DP) which were purchased from Cascade Biologics. HDF cells were cultured in Fibroblasts Growth Medium. Melanocytes were cultured in the growth medium M-254 which was supplemented with HMGS-2 as well as antibiotics.
The cells were preincubated in the appropriate growth medium at 5% CO_2 humidity and 37°C. Subsequently, one of the following procedures was applied:

Model 1: the medium was replaced by CPZ solutions in PBS with calcium and magnesium (PBS^{++}) and incubated for 1 h. Then the cells were irradiated with the **sunlight simulator SXL-3000V4** (UVA dose: 5 J/cm^2). The cells were then incubated for 24 h in the appropriate medium until analysis.

Model 2: the medium was replaced by CPZ solutions in medium and the cells were incubated for 23 h. Subsequently, CPZ solutions in PBS with calcium and magnesium (PBS++) was added for 1 h incubation and then the cells were irradiated with the **sunlight simulator SXL-3000V4** (UVA dose: 5 J/cm^2). The cells were then incubated for 24 h in the appropriate medium until analysis.

Cells proliferation screening assay

The proliferation of normal skin cells was estimated by WST-1 colorimetric assay. In brief, WST-1 is a tetrazolinum salt, which is transformed to formazan by metabolically active cellular mitochondrial dehydrogenases. The reagent was added to cells cultured in 96-well microplates in an amount of 10 µL/well 3 h before the measurement.

Statistics

Statistical analysis was performed using GraphPad Prism 7. Data are presented as mean values ± SD of three independent experiments in at least three repetitions The results were analyzed statistically using one-way ANOVA and two-way ANOVA, as well as Dunnett's and Tukey's multiple comparison tests. $p < 0.05$ was considered to indicate a statistically significant difference.

Conclusions

- ✓ **It was demonstrated that the extension of incubation time with CQ augments the drug phototoxic potential reflected by an increase in PIF values.**
- ✓ **A dramatic difference was observed between the cell types tested – fibroblasts were found to be significantly more sensitive to the phototoxic effect of CQ compared to melanocytes.**
- ✓ **The results indicate that the phototoxicity of CQ depends on cell type and drug exposure time. Therefore, optimal in vitro models used to evaluate the phototoxic potential of melanin-binding compounds should include cultures of cells with different pigmentation. Moreover, the preincubation time of 1 h, which is currently considered the standard, should be extended.**

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