IN VITRO MODEL STUDIES OF CHLOROQUINE-INDUCED PHOTOTOXICITY USING NORMAL SKIN CELLS - FIBROBLASTS IRRADIATED WITH A SUNSHINE SIMULATOR

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Background

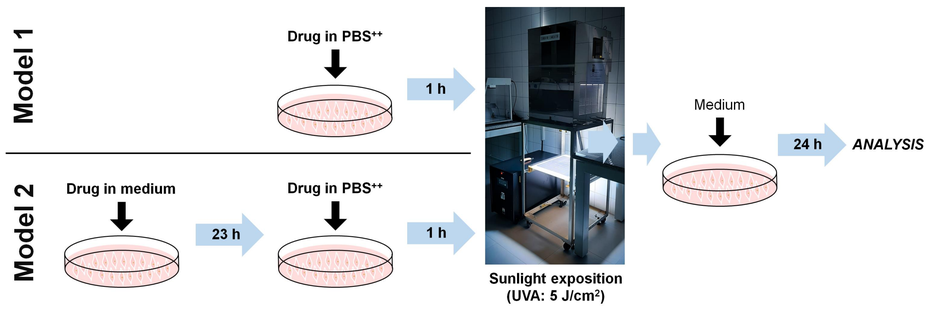
Abstract: Phototoxicity is an abnormal skin reaction induced by sunlight exposure in patients taking particular medicines. The phototoxic reactions occur as a result of the simultaneous interaction of two factors: exposure to UV radiation and the presence of a photosensitizer. Chemical compounds with the phototoxic potential that have the ability to absorb the energy of light radiation which may lead to compound degradation and induction of oxidative damage of cells. The number of photosensitization cases is constantly increasing.

One of the phototoxic drugs is chloroquine (CQ), which belongs to the group of protozoal drugs and is used to treat malaria. The drug is also used to treat some autoimmune, dermatological, vascular, and extra-intestinal amebiasis diseases. The use of chloroquine in the treatment of beta-coronavirus infections such as SARS-CoV, MERS-CoV and SARS-CoV-2 (believed to be the cause of COVID-19 disease) appears to be important. Due to its antiviral and antimicrobial properties, chloroquine has significant potential for persistence, bioaccumulation and translocation into living organisms in high-risk forms.

Aim of the study

The aim of this study was to assess changes in the homeostasis of human skin fibroblasts caused by phototoxic action of chloroquine. In order to implement the assumption of the study, the NRU test (cytotoxicity analysis) was performed. Additionally, the DNA fragmentation as well as reactive oxygen species were determined.

Two experimental models were applied, as presented below:



Methods

Cell culture and treatment

In vitro studies were performed on human dermal fibroblasts (HDF) obtained from Sigma-Aldrich The cells were cultured in Fibroblasts Growth Medium. HDFs 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.

NRU assay

The general assessment of cytotoxic and phototoxic potential were made using neutral red based In Vitro Toxicology Assay Kit (TOX4). Tested cells were seeded in 96-well plates and cultured for 48 hours. Then, they were treated according to experimental model 1 and 2. After that, 10 µL of 0.33% neutral red solution was added to each well. After 3-h incubation, the medium was removed and cells were rinsed with Neutral Red Assay Fixative. In the next step 100 µL of Neutral Red Assay Solubilization Solution was added to each well and the plates were stirred for 10 min at RT. The absorbance of the samples was measured at 540 nm with a reference wavelength of 690 nm using the microplate reader Infinite 200 PRO controlled by the Magellan software.

Intracellular level of reduced thiols

The measurement of the intracellular level of thiols was made using a fluorescence imaging cytometer NucleoCounter® NC-3000. The assay is based on VitaBright-48 - a highly specific dye staining cells with a high level of reduced thiols, e.g. GSH. Cell suspension was stained with Solution 5 (containing VitaBright-48, propidium iodide, and acridine orange) and analyzed with the image cytometer. The obtained histograms are used to differentiate the subpopulation of cells with high and low levels of reduced thiols.

DNA fragmentation assay

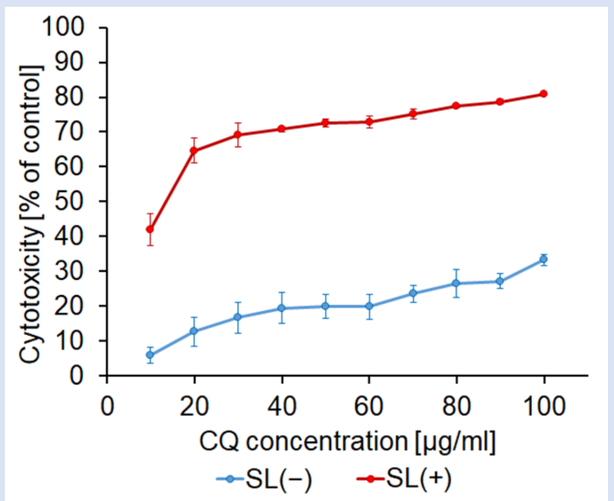
DNA fragmentation was assessed using the image cytometer. Following treatment, cells were counted, and fixed with ice-cold 70% ethanol. After washing, cell pellets were stained with solution containing DAPI and Triton X-100 and analyzed using the NC-3000 system.

Statistical analysis

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 or two-way ANOVA and Dunnett's or Tukey's test. $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Model 1



Model 2

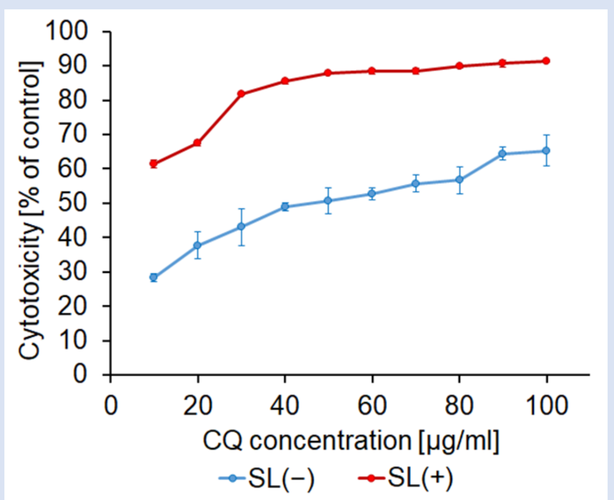
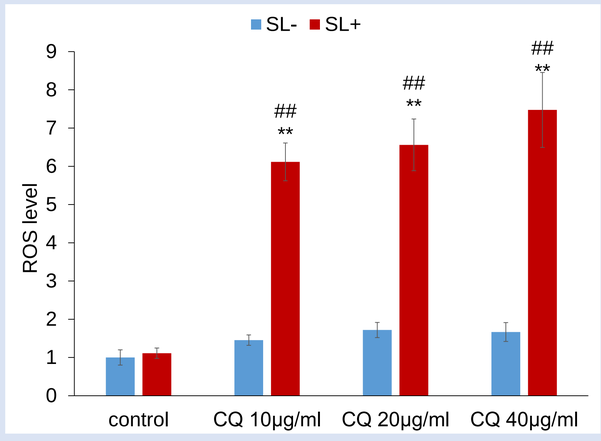


Figure 1.
Cytotoxicity of chloroquine (CQ) towards non-irradiated or exposed to sunlight (SL) human dermal fibroblasts. The cells were were treated as described in Materials and Methods and tested using the NRU assay. The results are demonstrated as the percentage of control.

Model 1



Model 2

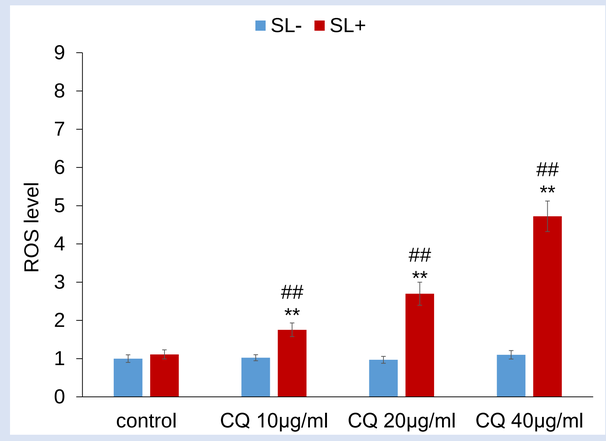
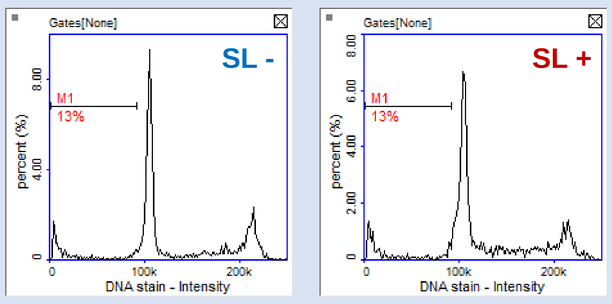


Figure 2.
Effects of chloroquine (CQ) on ROS level in non-irradiated or exposed to sunlight (SL) human dermal fibroblasts. The cells were treated as described in Materials and Methods. The results were expressed in units compared to control (control = 1) presented as mean values ± SD (**p<0.01 vs untreated control, ##p<0.01 vs cells exposed only to sunlight).

Model 1



Model 2

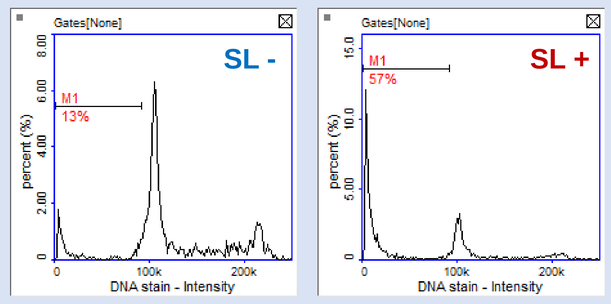


Figure 3.
Effects of chloroquine (20µg/ml) on DNA fragmentation of non-irradiated or exposed to sunlight (SL) human dermal fibroblasts. The cells were treated as described in Materials and Methods. DNA fragmentation was detected using the image cytometry. Representative histograms were shown (M1 – percent of cells with fragmented DNA).

Conclusions

- ✓ **It was found, that CQ cytotoxicity depends on the preincubation time. Extending the exposure of cells to CQ to 24 h revealed the drug cytotoxic potential. The irradiation augmented the drug-induced toxic effect significantly. Moreover, the increase in the amount of reactive oxygen species in samples exposed to the drug and UV radiation at both tested incubation times was demonstrated.**
- ✓ **The results obtained shows strong phototoxic properties of CQ towards fibroblasts, which is associated with the disturbance of the oxidation-reduction balance in the cells.**

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