OXIDATION-REDUCTION IMBALANCE IN CHLORPROMAZINE-INDUCED PHOTOTOXICITY - IN VITRO STUDIES ON DERMAL FIBROBLASTS IRRADIATED WITH A SUNSHINE SIMULATOR

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INTRODUCTION

Drug-induced phototoxicity is associated with the induction of oxidative stress. Drugs with phototoxic potential include, among others, phenothiazines. Under UV irradiation, phenothiazines are converted into photoproducts that can react with molecular oxygen, causing the production of reactive oxygen species. In addition, it has been shown that phenothiazines can accumulate in the skin, which may have a significant impact on the cutaneous adverse reactions induced by these drugs.

AIM OF THE STUDY

The aim of the study was to investigate the phototoxic potential of chlorpromazine (CPZ) - a phenothiazine derivative used in the treatment of schizophrenia and acute psychosis. According to the European Commission recommendations for in vitro phototoxicity testing, CPZ should be used as a model phototoxic substance. The studies were conducted using human dermal fibroblasts (HDF) irradiated with the sunlight simulator SXL-3000V4. Taking into account the possibility of drug accumulation in cells, fibroblasts were incubated with CPZ for 24 h or 1 h before the exposure to the sunlight.

MATERIALS AND METHODS

CELL CULTURE AND TREATMENT

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

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 1h. Then the cells were irradiated with the sunlight simulator SXL-3000V4 (UVA dose: 5 J/cm^2). Then, the cells were incubated for 24h in the medium until analysis.

Model 2: the medium was replaced by CPZ solutions in medium and the cells were incubated for 23h. Subsequently, CPZ solutions in PBS++ was added 1h before the irradiation with the sunlight simulator SXL-3000V4 (UVA dose: 5 J/cm^2). Then, the cells were incubated for 24 h in the appropriate medium prior the analysis.

Sunlight simulator SXL-3000V4

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.

ROS LEVEL ANALYSIS

The oxidation of 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA) into 2,7-dichlorofluorescein (DCF) was used to assess the ROS generation in cells after the treatment. In brief, 5000 cells per well were placed in a 96-well dark microplate and incubated at 37°C and 5% CO_2 for 24 h. After incubation, the medium was removed and the cells were treated according to model 1 or model 2. Then, the medium was removed and the cells were incubated with 10 M H_2DCFDA for 30 min at 37°C and washed twice with PBS to remove excess dye. The fluorescence was read at wavelengths of 485 nm of excitation and 530 nm of emission using a microplate reader Infinite 200 Pro (Tecan, Switzerland). The obtained results, normalized to a number of living cells, were finally expressed as a percentage of the controls.

STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism 7. 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 AND CONLUSIONS

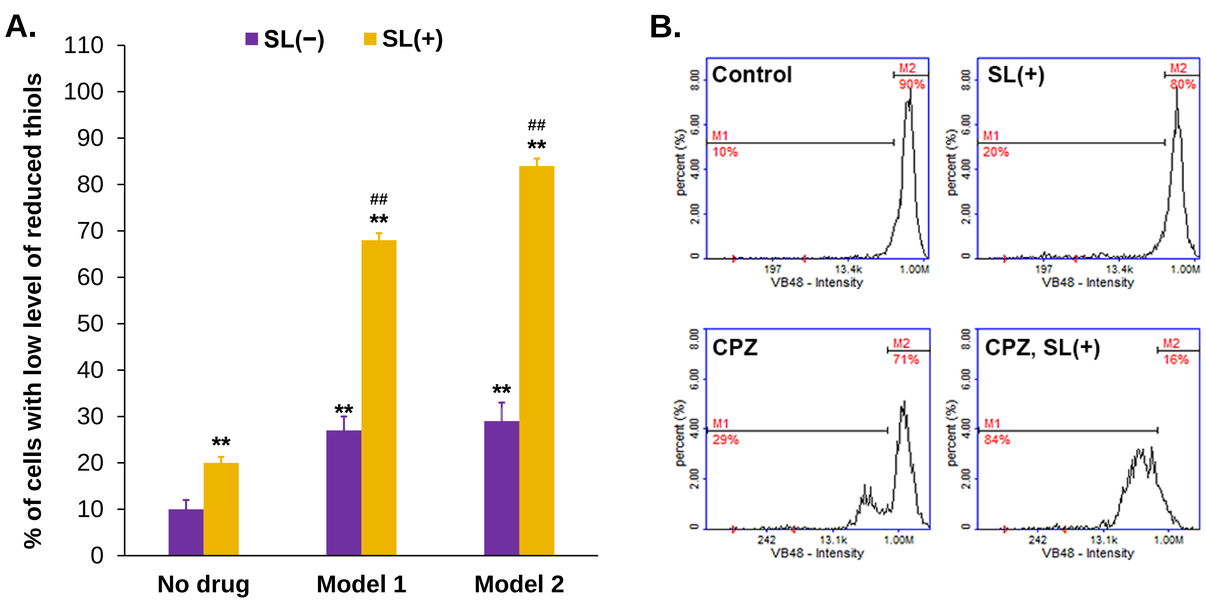

Figure 1. Effects of chlorpromazine (CPZ) in a concentration 5 μg/ml on intracellular thiols status of non-irradiated or exposed to sunlight (SL) human dermal fibroblasts. The cells were treated as described in Materials and Methods. The status of cellular thiols was analyzed using the image cytometry. (A) The results expressed as mean values ± SD (**p<0.01 vs untreated control, ##p<0.01 vs cells exposed only to sunlight). (B) Representative histograms obtained for CPZ in an experimental model 2 (M1 – percent of cells with low level of reduced thiols, M2 – percent of cells with high level of reduced thiols).

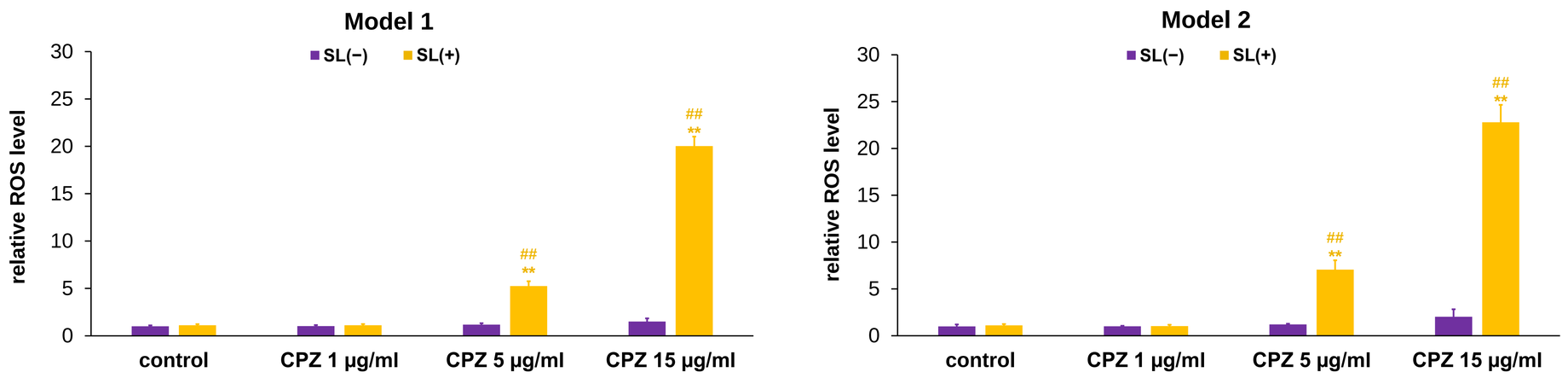

Figure 2. Effects of chlorpromazine (CPZ) on ROS level in non-irradiated or exposed to sunlight (SL) human dermal fibroblasts. The cells were treated as described in Materials and Methods. The results were expressed in units compared to control (control = 1) presented as mean values ± SD (**p<0.01 vs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

CONCLUSIONS

- **It was found, that exposure of cells to sunlight and chlorpromazine resulted in a significant increase in the level of reactive oxygen species and a decrease in the level of reduced cellular thiols. Changes in the oxidation-reduction state were more noticeable in cells treated with the drug 24 h before irradiation.**
- **The obtained results indicate that the phototoxic potential of chlorpromazine in terms of oxidoreductive imbalance disturbances depends on the exposure time to the drug.**

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