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Perphenazine and prochlorperazine induce concentration-dependent loss in human glioblastoma cells viability

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Received July 29, 2017, accepted September 7, 2017

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Pharmazie 73: 19–21 (2018) *doi: 10.1691/ph.2018.7806*

Phenothiazine derivatives possess biological properties very useful for cancer therapy, such as antiemetic and sedative activity as well as good blood–brain barrier permeability. Our goal was to determine if perphenazine and prochlorperazine are possessing cytotoxic activity towards U87-MG cells. It has been shown that the analyzed drugs induce concentration-dependent loss in cell viability, what correlates with their chemical structure. The calculated EC_{50} values for perphenazine (0.98 μM) and prochlorperazine (0.97 μM) are related to their toxic concentrations in human plasma. The obtained results suggest that perphenazine and prochlorperazine may have a potential for the development of new and effective anticancer therapies.

1. Introduction

Glioblastoma multiforme (GBM) is a common, primary and malignant type of brain tumors, with an annual incidence from 4.67 to 5.73 per 100,000 individuals and an average survival time of 12.1 (temozolomide and radiation) or 14.6 (radiation) months (Ostrom et al. 2014). Since GBM infiltrates surrounding tissues, its complete resection is impossible and radiotherapy is usually not efficient. Currently, the treatment of GBM involves surgery followed by external-beam radiation and concomitant temozolomide (TMZ) chemotherapy followed by additional 6 cycles of TMZ administration (Urbańska et al. 2014). What is more, the presence of blood-brain barrier limits penetration of different substances and drugs into the brain, thus alternative drug delivery strategies are required for a more effective treatment of glioblastoma (Jovčevska et al. 2013). Interestingly, phenothiazine derivatives possess various biological activities such as sedative, antiemetic (Motohashi et al. 2006; Sudeshna and Parimal 2010), anticancer activity(Jaszczyszyn et al. 2012; Girly et al. 2014) and good blood–brain barrier permeability (Bulic et al. 2009).

The aim of this work was to assess the effect of perphenazine and prochlorperazine on viability of human glioblastoma multiforme U87-MG cells. Moreover, we examined the effect of chlorpromazine, the oldest and the most representative phenothiazine derivative, on human glioblastoma viability.

2. Investigations and results

Cell viability was determined by the WST-1 assay. It has been demonstrated for the first time that U87-MG cells treated for 24 h with perphenazine as well as prochlorperazine in concentrations 0.5, 1, 5 and 10.0 μM lost 32, 54.5, 82.1 and 92.2 % as well as 30.5, 56.3, 84 and 92.7 % in cell viability, respectively (Fig. 1A). After 24-h incubation with chlorpromazine in concentrations 20, 50, 75 and 100 μM glioblastoma cells lost 13.1, 38.1, 77.9 and 97.1% of cell viability (Fig. 1B). At the lowest concentration of perphenazine and prochlorperazine (0.1 μM) as well as chlorpromazine (10 μM), no loss in cell viability was observed.

The EC_{50} values (the concentration of a drug that produces loss in cell viability by 50%) for chlorpromazine, perphenazine and prochlorperazine were calculated to be 45.61 μM, 0.98 μM and 0.97 μM, respectively.

3. Discussion

In this study we used the U87-MG cell line as an *in vitro* experimental model, because it is commercially available and very frequently used to test the cytotoxic activity of antitumor agents (Urbańska and Mandal 2014). It has been demonstrated that perphenazine and prochlorperazine induce concentration-dependent loss in glioblastoma cells viability (Fig. 1A). Up to now, only the viability of human glioma U87-MG cells treated with chlorpromazine, fluphenazine and thioridazine were examined. Shin et al. (2013) showed a significant decrease in cell viability in U87-MG cells treated for 24 h with high concentrations of chlorpromazine (≥20 μM). In lower concentrations, no loss in cell viability was observed (Shin et al. 2013), what confirms our results (Fig. 1B). Cheng et al. (2015) demonstrated a significantly higher cytotoxic effect of thioridazine (concentration of 10 μM) than of fluphenazine on human glioma U87-MG cells after 72 h treatment. Interestingly, all the results suggest that phenothiazine derivatives may inhibit glioblastoma cells viability in concentrations related to their lethal human plasma concentrations, which are 3 to 12 μg/ml (i.e. 8.44-33.77 μM) for chlorpromazine and 1 to 18 mg/ml (i.e. 2.46-44.22 μM) for thioridazine (Winek et al. 2001; Schulz and Schmoldt 2003). In opposite to those findings, the EC_{50} values obtained in this study indicate that perphenazine and prochlorperazine are significantly more cytotoxic towards U87-MG cells than chlorpromazine and correlate with their toxic concentrations in human plasma, which are from 0.05 to 1 μg/ml (i.e. 0.12-2.47 μM) for perphenazine and from 0.2 to 1 μg/ml (i.e. 0.33-1.65 μM) for prochlorperazine (Winek et al. 2001; Schulz and Schmoldt 2003). Taking into account the obtained EC_{50} values, the order of drugs cytotoxicity towards human glioblastoma cells is as follows: prochlorperazine ≥ perphenazine >> chlorpromazine.

In contrast to chlorpromazine with an aliphatic chain at position N-10, perphenazine and prochlorperazine possess a piperazinyl ring at this position (Fig. 2) (Sudeshna and Parimal 2010). Comparison of chemical structure and *in vitro* cell viability data suggests that the piperazinyl substituent at N-10 position is very important for phenothiazine derivatives cytotoxicity. It is noteworthy that the drug with aliphatic chain demonstrates a significantly lower anticancer activity. Thus, chemical diversity may probably explain the differences in cell viability of human glioblastoma treated with phenothiazine derivatives.

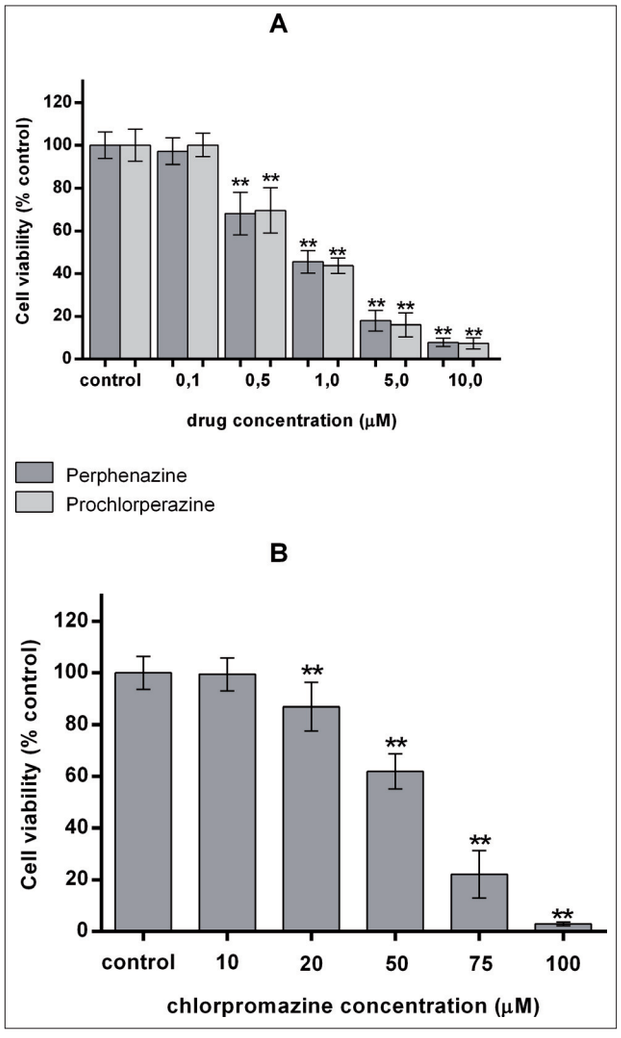

Fig. 1: The effect of perphenazine and prochlorperazine (A) as well as chlorpromazine (B) on human glioblastoma cells viability. Cells were treated with various perphenazine and prochlorperazine (0.1–10.0 μM) as well as chlorpromazine (10.0–100.0 μM) concentrations and examined by the WST-1 assay. Data are expressed as % of the controls. Mean values ± SD from three independent experiments (n=3) performed in triplicate are presented. ** $p < 0.01$ vs. the control samples.

Our results demonstrate for the first time the cytotoxic activity of perphenazine and prochlorperazine towards U87-MG cell line, which is much higher than that of chlorpromazine and may be explained by differences in the chemical structure of these compounds. The obtained EC_{50} values for perphenazine and prochlorperazine correlating with their toxic human plasma concentrations suggest that both drugs are safer than chlorpromazine and *may* have the potential for the development new and effective anticancer therapies. Our results also confirm that the U87-MG cell line is a suitable cell model to study cytotoxic activity of new antitumor agents.

4. Experimental

4.1. Chemicals

Chlorpromazine hydrochloride, perphenazine, prochlorperazine dimaleate, amphotericin B and penicillin G were purchased from Sigma-Aldrich Inc. (USA). Cell Proliferation Reagent WST-1 was acquired from Roche GmbH (Germany). Neomycin sulphate was obtained from Amara (Poland). Growth medium DMEM, fetal bovine serum (FBS) and trypsin/EDTA were obtained from Cytogen (Poland).

4.2. Cell treatment

The human glioblastoma cells U87-MG (Sigma Aldrich) were cultured in DMEM basal medium supplemented with FBS (10%), neomycin (10 μg/mL), amphotericin B (0.25 μg/mL) and penicillin G (100 U/mL) at 37 °C in 5% CO_2.

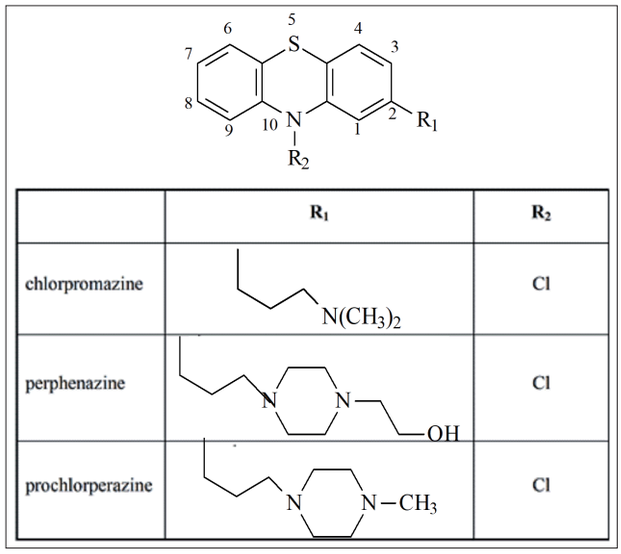

Fig. 2: The structure of chlorpromazine, perphenazine and prochlorperazine (Sudeshna and Parimal 2010).

4.3. Cell viability assay

The viability of melanocytes was evaluated by the WST-1 (4-(3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulphonate) colorimetric assay according to the method described earlier (Beberok et al. 2011; Otręba et al. 2015) with slight modification. U87-MG cells were seeded in 96-well microplate at a cell density of 2500 cells/well and incubated in supplemented DMEM growth medium for 24 h. The absorbance of the samples was measured using a microplate reader Tecan Infinitie M200 Pro (Tecan group Ltd., Switzerland).

4.4. Statistical analysis

In all experiments, mean values of at least three separate experiments (n=3) performed in triplicate±standard deviation (SD) were calculated. Statistical analysis was performed with one-way ANOVA followed by Tukey post-hoc test using GraphPad Prism 6.01 Software. The significance level was estabilished at value of $p<0.01$ (**), by comparing the data with those for the control (cells without drug).

Acknowledgments: This work was financially supported by the Medical University of Silesia in Katowice, Poland (Grant No. KNW-012-N/6/N).

Declaration of interest: The authors: Michał Otręba and Ewa Buszman declare that there is no conflict of interest. The manuscript entitled: ''Perphenazine and prochlorperazine induce concentration-dependent loss in human glioblastoma cells viability", which all the listed authors have read and approved, has not been submitted elsewhere for publication, in whole or in part, and is permitted to be published in this journal.

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